Final report

Molecular risk assessment of Salmonella in red meat

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Abstract

The U. S. Department of Agriculture (USDA) have expressed their desire to reduce *Salmonella* infections, accelerating initiatives to achieve 25% reduction by 2030. In one initiative, the USDA have begun developing tools for identifying *Salmonella* serovars that present the highest risk to humans, referred to as Highly Pathogenic *Salmonella* (HPS). Here, we assess whether Australian red meat *Salmonella* align with HPS and conduct genomic analyses with publicly available sequences from Australian *Salmonella* of clinical relevance.

Except for *S.* Typhimurium, few *Salmonella* spp. from Australian red meat sources belonged to the major HPS serovars (Dublin, Enteritidis, Newport, Typhimurium, I 4,[5],12:1). Relationship assessments show that some serovars clustered mostly by host (animal or human), while other serovars did not. Several virulence genes were serovar associated and while most isolates from the same serovar had identical virulence gene profiles, a small number of genes were differentially associated with isolates of animal or human origin.

Antimicrobial resistance (AMR) genes were detected in very low percentages of Australian *Salmonella*. The project provides initial genomics insight into Australian *Salmonella* and the outcomes will serve as a valuable resource in risk assessment and management of *Salmonella* in Australian and global supply chains.

Executive summary

Background

The U. S. Department of Agriculture (USDA) has recently accelerated efforts to meet a self-imposed 2030 target of reducing *Salmonella* infections associated with Food Safety and Inspection Service (FSIS) regulated products by 25%. In a major step towards achieving this goal, the USDA has announced their intent to declare *Salmonella* an adulterant of certain poultry products. In a similar vein, the Marler Clark Food Safety Law Firm, has petitioned the FSIS to declare 31 serovars of *Salmonella* adulterants of meat and poultry products in the US and the U.S. Meat Animal Research Center (USMARC), a branch of the USDA, has begun developing new diagnostic tools for the detection and quantitation of high-risk *Salmonella (HRS)*. While there is no indication of intent by the FSIS to regulate *Salmonella* in beef, this project set out to perform an initial genomics assessment of Australian *Salmonella* alignment with the major disease causing serovars in the US.

Objectives

The project was designed to provide initial genomics insights into Australian red meat and human derived *Salmonella*. The objectives of the project were (i) to determine if Australian isolates align with the major disease-causing serovars identified in US human cases which are referred to as highly pathogenic *Salmonella* (HPS) or DENT (i.e. Enteritidis, Typhimurium, 1,4,[5],12: i:-, Newport, and the invasive serovar Dublin) and (ii) conduct genomic analyses, including comparisons with publicly available *Salmonella* sequences to determine if *Salmonella* from Australian red meat supply chains align with *Salmonella* isolated from Australian human clinical cases of salmonellosis.

Methodology

A flexible, version management capable and secure workflow was developed for the rapid, high throughput computational analysis of *Salmonella* whole genome sequences. A large representative set of *Salmonella* isolates from beef, goat and sheep housed within the CSIRO culture collection were selected for the study and included *Salmonella* of Australian public health concern, those which belong to DENT or those belonging to other HPS serovar groups. The balance of the subset comprised isolates from diverse sources (e.g. hide, oral, rumen) or serovars of prominence globally. Selected isolates were sequenced and compared with regionally relevant, publicly available collections of *Salmonella* through genome-wide comparisons of *Salmonella* Pathogenicity Islands (SPI), phylogenetic assessments of strain relatedness, comparisons of the virulome (virulence genes) and resistome (AMR genes).

Results/key findings

Except for *S.* Typhimurium, few *Salmonella* from Australian red meat sources belonged to the major HPS serovars (DENT). Phylogenetic trees showed that some serovars of Australian *Salmonella* isolates clustered mostly by host (animal or human for *S.* Typhimurium, *S*. Anatum and *S.* Infantis), while no obvious distinction between animal and human isolates was observed for the other serovars (*S.* Saintpaul and *S.* Bovismorbificans). Several virulence genes were shown to be highly associated with some serovars but not others. Isolates from the same serovar mostly had similar virulence gene profiles, regardless of source (animal vs human), however, a small number of genes were shown to be more prevalent in humans, while others were more prevalent in animals. AMR genes were detected in very low percentages of the *Salmonella* isolates, with most AMR genes absent from most animal derived strains.

Benefits to industry

The database of *Salmonella* sequences, genomics capability and workflows developed in this project will serve as a valuable resource for industry to better understand and manage risks associated with *Salmonella*. In addition, data can be used to inform risk management practices that in turn minimise the reputational, trade and public health risks associated with contaminated beef entering domestic and export markets.

Future research and recommendations

In the absence of international standards, we recommend assessing the performance of bioinformatics-based typing methods against traditional 'gold standard' characterisation tools or commercial test methods for identifying HPS (when / if they become available). Additional surveys of *Salmonella* could be undertaken on animals across a broader range of different sample sites (e.g., hides and lymph nodes) to identify potential carcase contamination pathways for HPS. Further attempts should be made to establish collaborations with US-based researchers to access propriety information for deeper assessment of HPS and DENT serovars. Comparison of Australian HPS with international HPS should be conducted. Collaborations should be pursued with local public health labs to gain access to metadata sets for improved risk characterisation. When/if available, rapid systems should be trialled for the semiquantitative or quantitative detection of high-risk *Salmonella*.

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1. Background

The Australian red meat industry continues to be a significant contributor to the economy with $18.4 B in export and $13.4 B in domestic sales, which is underpinned by a reputation for producing clean, green and safe red meat products. Continued awareness and adoption of improved methods of understanding and managing food safety risks will support the ongoing profitability and sustainability of the industry. Recent developments in the US have highlighted the importance of managing *Salmonella* in meat production systems, particularly poultry, and there's potential to introduce new performance standards in the US for high-risk meat products.

Although there are 1,547 serovars of *Salmonella* enterica (1), epidemiological data points to a limited number of serovars as the cause of most human illness. In an effort to reduce outbreaks, the Marler Clark Food Safety Law Firm has, on behalf of others, petitioned the US Food Safety and Inspection Service (FSIS) to declare 31 outbreak-associated serovars adulterants of meat and poultry in the US (2). In a similar effort to support food safety management, the US Meat Animal Research Center (USMARC) have begun developing methods to rapidly identify the presence and concentration of virulent strains of *Salmonella* (Figure 1), the results of which have enabled the USMARC to define groups of *Salmonella* as HPS (highly pathogenic *Salmonella*) or nominate specific serovars (i.e., DENT: Dublin, Enteritidis, Newport, Typhimurium) of greatest importance (3). The need to develop new tools to assist existing risk management practices is supported by a growing body of evidence from recalls of large volumes of ground beef due to the presence of *Salmonella* and multistate outbreaks of salmonellosis in the US.

	Serotype size (bp)	n	HPS-6	HPS-1	HPS-3	HPS-5	HPS-4	HPS-2	invA	number of targets detected
DENT	**Typhimurium**	**171**	99.4	28.1	100.0	100.0	69.6	100.0	100.0	5-7
DENT	Dublin	44	4.5	100.0	100.0	95.5	88.6	100.0	100.0	5-6
DENT	Enteritidis (80%)	24	0.0	8.3	100.0	100.0	100.0	100.0	100.0	5-6
DENT	**Newport (93%)**	**172**	100.0	2.3	100.0	100.0	0.0	99.4	100.0	5-6
DENT	1,4,[5],12:i:-	14	100.0	0.0	100.0	100.0	0.0	100.0	100.0	5-6
	Heidleberg	22	100.0	4.5	100.0	100.0	0.0	0.0	100.0	4
	Newport* (7%)	12	100.0	0.0	83.3	100.0	0.0	16.7	100.0	3-4
	Lubbock	65	100.0	0.0	98.5	100.0	0.0	0.0	100.0	3-4
	Mbandaka	39	97.4	0.0	100.0	100.0	0.0	0.0	100.0	3-4
	Infantis	19	78.9	0.0	94.7	94.7	0.0	15.8	100.0	3-4
	Muenchen	12	91.7	0.0	91.7	91.7	0.0	0.0	100.0	3-4
	Thompson	11	9.1	9.1	90.9	90.9	0.0	54.5	100.0	3-4
	Kentucky	56	60.7	0.0	96.4	94.6	0.0	0.0	100.0	3-4
	Enteritidis* (20%)	6	0.0	0.0	66.7	83.3	0.0	83.3	100.0	3-4
	Meleagridis	32	0.0	0.0	100.0	100.0	0.0	0.0	100.0	3
	Agona	28	0.0	0.0	100.0	100.0	0.0	0.0	100.0	3
	Anatum	**288**	100.0	0.0	100.0	0.0	0.0	0.0	100.0	3
	Lille	46	100.0	0.0	0.0	100.0	0.0	0.0	100.0	3
	Reading	16	87.5	0.0	12.5	75.0	0.0	12.5	100.0	3-4
	Montevideo* (4.5%) Clade IV	7	28.6	14.3	100.0	28.6	0.0	14.3	100.0	3-4
	Cerro	**205**	51.7	0.0	100.0	21.0	0.0	0.0	100.0	2-3
	Muenster	59	6.8	0.0	6.8	0.0	0.0	10.2	100.0	2-3
	Montevideo (95.5%) Clade I	**148**	0.0	0.0	0.0	0.0	0.0	0.0	100.0	1
	EB (39); GB Enrichments (373)	**412**	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0
	Total samples tested:	**1908**								

Table 1. Reprint of a proposed approach for identifying Highly Pathogenic *Salmonella* (HPS) serovars as it appears in published work by the US Meat Animal Research Center (USMARC) (3). HPS serovars are noted for being highly invasive in humans or for their association with human disease in the US and are identified by the presence of genetic targets (Highly Pathogenic *Salmonella*, >3 targets). Non-HPS are predicted to have lower potential to cause human disease (≤ 3 targets).

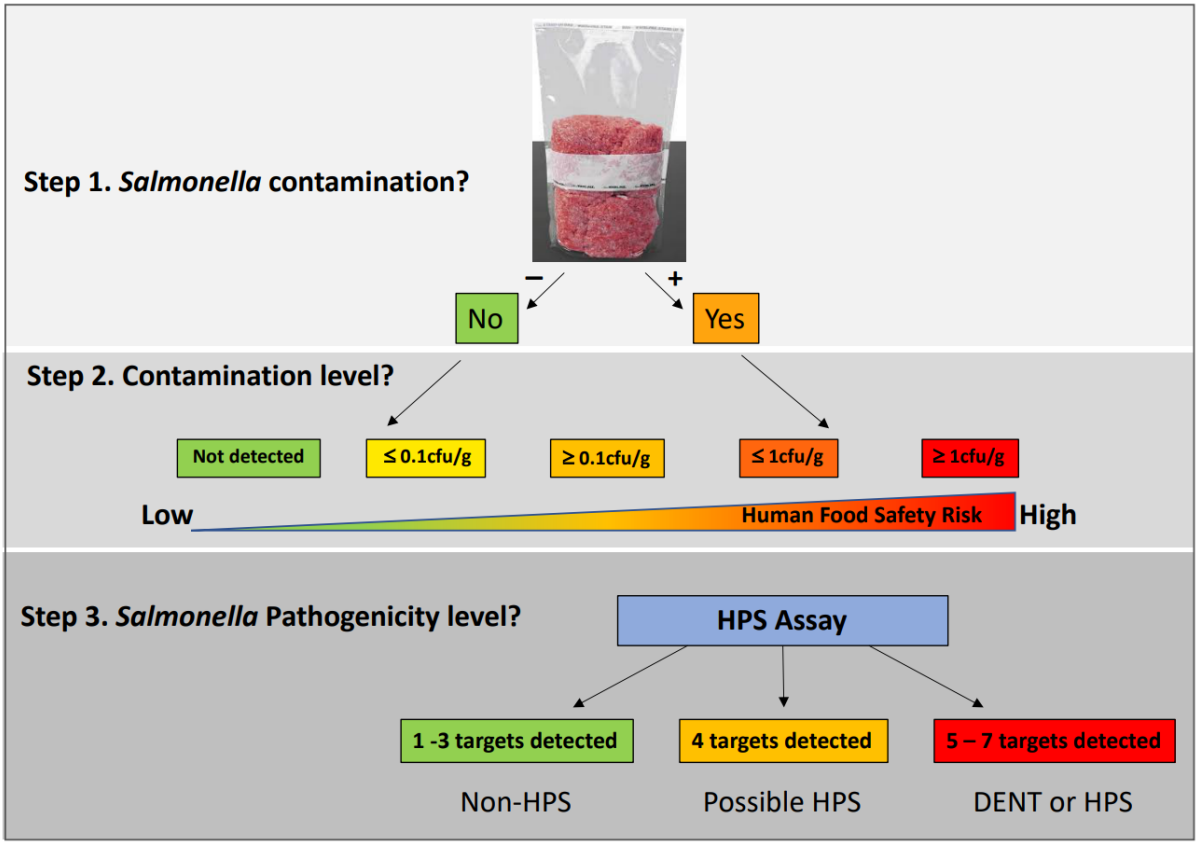

Figure 1. Reprint of an illustration, as it appears in a publication from the US Meat Animal Research Center (USMARC) (3), of a hypothetical quantitative approach for mitigating risks associated with Highly Pathogenic *Salmonella* serovars in foods.

While the USMARC approach may be used to aid existing practices to manage *Salmonella* in red meat, there is no indication of imminent adoption by regulators for red meat applications. In contrast, the FSIS have recently announced a proposal to declare *Salmonella* an adulterant in breaded stuffed raw chicken products when present at concentrations above a defined level (4). The proposed declaration by the USDA is the first of multiple actions that will be taken to reduce the rate of salmonellosis in the US and initially targets poultry due to the high percentage of *Salmonella* infections in the US which can be attributed to poultry consumption (4).

The Australian red meat industry has made assessments of *Salmonella* prevalence and concentration in national pathogen surveys (5, 6), baseline studies (7, 8) and through the National Carcase Microbiological Monitoring Program. Isolates recovered from several of these studies have also been assessed for antimicrobial resistance and serotyped to determine serovar prevalence. However, studies which genotypically characterise *Salmonella* from Australian red meat animals are limited. This gap in data precludes the industry from developing an understanding of how their product might perform if alternate performance standards are adopted in international markets. The purpose of this study was therefore to (i) determine if Australian *Salmonella* from red meat and clinical sources align with priority *Salmonella* serovars in the US, and (ii) determine if *Salmonella* isolated from Australian red meat production systems align with *Salmonella* that have caused human disease in Australia.

2. Objectives

1. Determine if Australian isolates align with the major disease-causing serovars identified in US human cases which are referred to as highly pathogenic *Salmonella* (HPS) or DENT (i.e. Enteritidis, Typhimurium, 1,4,[5],12: i:-, Newport, and the invasive serovar Dublin.

2. Conduct genomic analyses, including comparisons with publicly available *Salmonella* sequences to determine if *Salmonella* from Australian red meat supply chains align with Australian human clinical cases of salmonellosis.

The objectives of this project were met, and the details of achievements are described in the subsequent sections. In brief, the CSIRO culture collection was interrogated for *Salmonella* serovars that align with the priority serovars outlined by the USMARC (Figure 1), the Marler Clark petition to the FSIS (2), and those that are of clinical significance in Australia. Isolates meeting this definition were sequenced and comparative genomic analyses were performed against Australian clinical isolates that have previously caused salmonellosis. Attempts were made to access international data to assess Australian *Salmonella* against methods proposed by the USMARC, however, due to potential commercialisation of the proprietary information we were unable to access this data. For this reason, investigations to determine if Australian isolates align with HPS were limited to assessing the occurrence of DENT in Australian production systems.

3. Methodology

3.1 Selection of isolates and sequences for genomic comparisons

Salmonella isolates were selected from the CSIRO culture collection for inclusion in the study. The strain set was selected to represent isolates recovered from previous studies of beef cattle, sheep and goat, with preference given to isolates recovered from large national surveys. Selection criteria were employed that preferenced *Salmonella* serovars of Australian public health concern, those that belong to DENT (i.e., Typhimurium, Dublin, Enteritidis, Newport and 1,4,[5],12: i:-), and those that are listed in FSIS docket No. FSIS-2020-0007. The balance of the subset comprised isolates from diverse sources (i.e., hide, oral, rumen) or serovars of prominence globally.

3.1.1 Selection of Australian *Salmonella* isolates from cattle sources

A total of 598 isolates were selected from the CSIRO collection for inclusion in WGS analyses (Table 2). To ensure that the project target of 500 isolates was achieved, an additional 98 isolates were included to account for conflicting serovar identification (conventional vs sequence serotyping), misidentification of original serovars and sequences that fail quality control during processing or analysis.

Isolates were selected from a range of projects spanning 2001 and 2019 (Figure 2) on the basis that they were isolated from red meat industry associated samples, that they had serovar data available and that they met the following requirements:

- DENT - isolates belonging to the "Highly Pathogenic *Salmonella*" serovar groupings: Typhimurium, Dublin, Enteritidis, Newport and 1,4,[5],12: i:-.
- Marler - isolates representing serovars listed in the Marler petition to the US FSIS: docket No. FSIS-2020-0007
    - Preferencing cattle and sheep sources, particularly from national faecal surveys

  - Minimised pooled or environmental isolates.
  - Goat isolates selected to cover a larger variety of phage types where such data was available.
  - Goat isolates included if that serovar was not present in cattle or sheep isolate sets.
  - Prioritised more recent isolates where collection covered multiple years and where metadata had been collected.
- NNDSS – additional serovars representing Australian clinical associated serovars reported as part of the National Notifiable Diseases Surveillance System (NNDSS) (9), that were not already captured in DENT or Marler serovar lists.
  - Isolates were prioritised in the same manner as Marler isolates.
  - In addition to the Marler and DENT serovars, another 7 serovars were included to represent those of Australian public health concern. When combined with the Marler and DENT serovars, these account for 85 % of the most prevalent human associated serovars reported by the National Notifiable Diseases Surveillance System (NNDSS) between 2009 and 2020.

The *Salmonella* subset of 598 isolates comprises 425 (77% of all serotyped isolates) from cattle, 119 (87% of all serotyped isolates) from sheep and 54 (17% of all serotyped isolates) from goat sources. Isolates were prioritised if they had previously been isolated from national Australian surveys and/or where metadata was available that could be used to contextualise risk in subsequent analyses.

Table 2 – Number of serotyped *Salmonella* isolates of priority serovar groups (DENT, Marler, NNDSS) from the CSIRO culture collection from red meat sources.

	Total number of isolates	Number for inclusion	Cattle		Goat		Sheep	
			Total number for inclusion - %[1]		Total number for inclusion - %		Total number for inclusion - %	
DENT	**206**	**186**	**144**	**100%**	**9**	**31%**	**33**	**100%**
S. Dublin	4	4	4	100%				
S. Enteritidis	1	1	1	100%				
S. Newport	1	1	1	100%				
S. Newport/S. Bardo	1	1	1	100%				
S. Typhimurium	199	179	137	100%	9	31%	33	100%
I 4,[5],12:i:- [2]	0	0						
Marler	**659**	**304**	**210**	**63%**	**41**	**15%**	**53**	**85%**
S. Agona	51	20	4	100%	4	15%	12	57%
S. Anatum	176	69	52	72%	7	7%	10	100%
S. Anatum var 15+ (Newington)	1	1	1	100%				
S. Derby	5	2			2	40%		
S. Heidelberg	39	8	2	100%	6	16%		
S. Infantis	35	31	15	100%	4	50%	12	100%
S. Infantis/Agona	1	1			1	100%		
S. Kottbus/Agona	1	1			1	100%		
S. Mbandaka	29	21	20	71%			1	100%
S. Montevideo	29	18	18	62%				
S. Muenchen	76	18	13	22%	2	14%	3	100%
S. Muenchen/S. Virginia	4	4	4	100%				
S. Oranienburg	4	4			4	100%		
S. Pakistan/S. Litchfield	1	1	1	100%				
S. Poona	3	3	3	100%				
S. Reading	25	23	13	87%			10	100%
S. Saintpaul	140	53	42	67%	8	11%	3	100%
S. Senftenberg	31	18	15	54%	2	100%	1	100%
S. Thompson	8	8	7	100%			1	100%
NNDSS	**148**	**108**	**71**	**90%**	**4**	**15%**	**33**	**79%**
S. Aberdeen	5	5	5	100%				
S. Bovismorbificans	79	66	33	100%	2	33%	31	78%
S. Chester	34	15	12	100%	2	10%	1	100%
S. Hvittingfoss	2	2	2	100%				
S. Singapore	2	2	1	100%			1	100%
S. Virchow	24	16	16	67%				
S. Wangata	2	2	2	100%				
Grand Total	**1013**	**598**	**425**	**77%**	**54**	**17%**	**119**	**87%**

[1]Number of isolates from each animal source and % for inclusion in WGS analysis

[2]Although I 4,[5],12:i:- has been isolated from human clinical cases in Australia, this serovar has not yet been isolated from Australian red meat in past CSIRO studies.

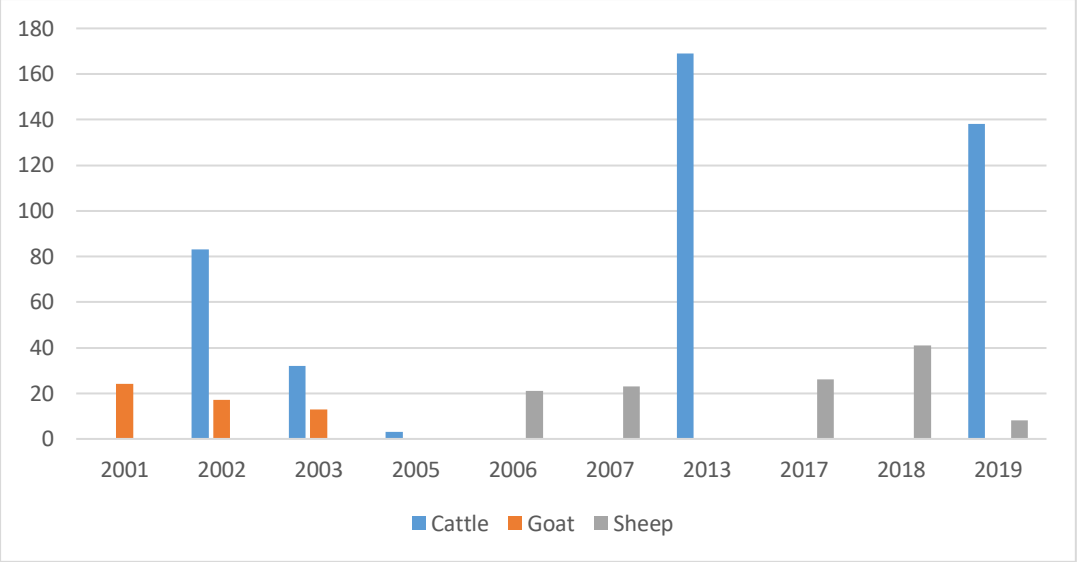

Figure 2. Number of *Salmonella* isolates included for WGS analysis from different red meat sources by year of isolation.

3.1.2 Selection of Australian *Salmonella* sequences from human sources

The National Center for Biotechnology Information (NCBI) is a popular online resource for storing biological sequence data. Much of the data is publicly available for use in genomics investigations, however, detailed information (origin, location of isolation etc) is often limited or omitted. In the current study, we utilised NCBI to access all *Salmonella* whole genome sequences (n=2972) that had been deposited as part of BioProject 319593 (as of 22 June 2023). BioProject 319593 contains sequences of *Salmonella* enterica isolates that are deposited to the database as part of routine surveillance activities conducted by the Melbourne Diagnostic Unit Public Health Laboratory. The database is predominantly comprised of sequences from *Salmonella* associated with human disease in Australia and while minimal metadata is available for submitted sequences, we believe this is a good starting point for selecting human isolate sequences for comparison with red meat isolates.

As serotyping data was not available for human isolate sequences, we conducted *in silico* analysis to predict serovars using the SeqSero2 tool (10). Sequences that were predicted to belong to *Salmonella* that align with the priority serovars described in section 3.1.3 (DENT, Marler, NNDSS) were combined with available metadata and a subset was selected for in-depth analysis as follows:

- Sequences from *Salmonella* that were isolated from non-human hosts were removed from analyses, as were those with missing host data.
- Clinical serovars were only included when they also occurred in the animal isolate list (i.e., those that matched priority serovars).
- Attempts were made to represent all years of collection for each serovar.
- Attempts were made to achieve similar numbers of human and animal sequences within each serovar.
- Where there were fewer clinical isolates than animal isolates, all clinical isolates were chosen.
- If the number of clinical isolates was greater than the number of animal isolates, clinical isolates were chosen from across all years of isolation and weighted against the number within each year.
- A small fraction of the total available clinical sequences from *Salmonella* Enteritidis were included as there was only one animal isolate and other sources (i.e., poultry) are well established for this serovar.

3.1.3 Preparation of Australian red meat associated *Salmonella* for whole genome sequencing

Genomic DNA (gDNA) extractions were prepared using the Gram-negative protocol of the DNeasy Blood and Tissue Kit (Qiagen) using a QIAcube automated purification system (Qiagen). Preliminary quality control was performed on gDNA using a Qubit Fluorometer (Thermofisher) with the dsDNA High Sensitivity Assay Kit (Invitrogen). Additional quality control of gDNA, library preparation and sequencing runs were performed at the Australian Centre for Ecogenomics, University of Queensland. DNA libraries were prepared using the Nextera DNA Flex library preparation kit (Illumina) and paired-end (2x150bp) reads were generated using the NovaSeq 6000 system (Illumina).

3.1.4 Bioinformatic analysis of sequences

A bioinformatics workflow was developed for the rapid, high-throughput computational analysis of *Salmonella* whole genome sequences, which incorporates the below steps. The workflow was developed in Galaxy - a workflow management tool that allows for scalable, sharable, and reproducible, version controlled computational workflows. CSIRO maintains its own instance of Galaxy that interacts with CSIRO's big data storage and high-performance computing facilities which collectively enable the rapid and high throughput risk profiling of isolates.

Sequence read files were concatenated where required. Illumina adaptors were removed as were low quality bases (clipping) using Fastp v0.20.1 (11) and outputs were visualised in MultiQC v1.9 (12). Reads were De Novo assembled using Spades version 3.12.0 (13) with kmers of 21, 33, 55, 77. Constructed assemblies were quality assessed with QUAST version 5.0.2 (14) and contiguous DNA segments (contigs) were screened for genes using Abricate (15) with the Virulence Factor database (16) for virulence gene determination and the Resfinder (17) database for antimicrobial resistance genes. Multilocus Sequence typing was performed using the MLST tool (Galaxy version v2.22.0) (18) with the PubMLST (19) *S.* enterica Achtman 2 scheme. The Abricate tool was also used to detect *Salmonella* Pathogenicity Islands (SPI) with the SPIFinder database located at the Center for Genomic Epidemiology (https://cge.food.dtu.dk/services/SPIFinder/). All Abricate outputs were filtered to achieve >80% base identity over >80% read length match with database entries. Any sequences not meeting these requirements were dropped from the analysis. All outputs were processed in Python version 3.10.9 to clean and transform data for generating results tables.

Phylogenetic analyses were performed to assess the relationship between animal and human isolates within the major *Salmonella* serovars included in the study. A total of five phylogenetic trees were constructed, one for each of the predominant serovars: *S*. Typhimurium (n=351), *S.* Anatum (n=130), *S.* Bovismorbificans (n=122), *S.* Saintpaul (n=111) and *S.* Infantis (n=63). The Center for Food Safety and Applied Nutrition (CFSAN) SNP pipeline (v 2.2.1) (20) was used to generate a SNP pairwise matrix, with the following reference strains for each serovar: *S.* enterica Typhimurium strain FDAARGOS_878 (CP065718.1), *S*. Anatum strain (GCF_001623625.1), *S.* Saintpaul strain (GCF_001952995.1), *S.* Bovismorbificans strain (GCF_018340585.1), *S.* Infantis (GCF_001931575.1). Distance matrix SNP alignment files were visualised with SplitsTree5 (21) using the NeghbourNet method prior to using RAxML (v 8.2.4) (22) to infer the maximum likelihood tree with the GTRCAT substitution model. The best-scoring ML Tree was then visualised using iTOL v6 (23) and displayed as mid-point rooted trees. Concentric rings were overlayed on tree diagrams to display the source of the isolate (host – inner ring), the collection year (middle ring) and the sequence type (outer ring).

4. Results

4.1 *Salmonella* isolate selection

As part of large-scale Australia wide surveys conducted by CSIRO between 2001-2019, there was a total of 1013 *Salmonella* isolates with serovar information available in the CSIRO culture collection. Of these, 206 (20.3%) isolates belonged to one of the DENT serovars - 199 (19.6%) were Typhimurium, 3 (0.3%) were Dublin, 3 (0.3%) were Newport and 1 (0.1%) was Enteritidis, and 0 (0%) were I 4,[5],12:i:-. Of the subset of 598 isolates sent for sequencing, 524 were confirmed *in silico* to belong to a priority serovar listed in Table 2. The number of isolates belonging to DENT serovars remained similar when assessed for serovar by *in silico* methods, confirming that DENT serovars, other than *S*. Typhimurium, have been infrequently isolated from red meat production sources in Australia.
Of the 2972 human *Salmonella* sequences obtained from the NCBI database, 1976 were predicted to belong to a priority serovar. To reduce high computational requirements and to make analysis more manageable, a subset of the 1976 human sequenced isolates was selected using the criteria outlined in section 3.1.2. The subset (557 sequences) was chosen to be of a similar size to that of the animal set, resulting in a total of 1,081 sequences for inclusion in subsequent comparative genomics assessments (Table 3).

Table 3. *Salmonella* serovars included in the study.

Predicted Serovar*	Cattle	Goat	Sheep	Animal Total	Human	Grand Total
Aberdeen	5	0	0	5	1	**6**
Agona	4	5	11	20	21	**41**
Anatum	45	6	9	60	70	**130**
Bovismorbificans	30	2	24	56	66	**122**
Chester	11	1	2	14	15	**29**
Derby	0	1	0	1	3	**4**
Dublin	3	0	0	3	17	**20**
Enteritidis	0	0	0	0	16	**16**
Gallinarum or Enteritidis	1	0	0	1	0	**1**
Heidelberg	3	5	0	8	5	**13**
Hvittingfoss	2	0	0	2	3	**5**
Infantis	15	5	6	26	37	**63**
Kottbus	2	1	0	2	0	**3**
Litchfield	1	0	0	1	1	**2**
Mbandaka	17	0	0	17	4	**21**
Montevideo	16	0	0	16	2	**18**
Muenchen	22	2	1	25	5	**30**
Newport	3	0	0	3	19	**22**
Poona	3	0	0	3	4	**7**
Reading	15	0	6	21	3	**24**
Saintpaul	49	8	1	58	53	**111**
Singapore	1	0	0	1	1	**2**
Thompson	0	0	0	0	8	**8**
Typhimurium	127	10	27	164	187	**351**
Virchow	15	0	0	15	16	**31**
Wangata	1	0	0	1	0	**1**
Grand Total	**391**	**46**	**87**	**524**	**557**	**1081**

*Serovar was predicted from whole genome sequences using the SeqSero2

4.2 Comparisons between *Salmonella* isolates from human and animal sources in Australia

4.2.1 Phylogenetic relationships of selected *Salmonella* serovars from Australian animal and human sources

The relationships between *Salmonella* isolates can be inferred from phylogenetic trees or network diagrams that are generated from SNP distance matrices (a measure of the DNA sequence differences between isolates in the analysis). Isolates that are closely related to each other will cluster together on the branches of the tree or network based on DNA sequence similarity.

The observation of a low level of reticulation in NeighbourNet analyses of distance matrix SNP alignments was used to support the validity of applying phylogenetic analysis rather than network analysis for assessing isolate relatedness.

Phylogenetic trees were generated for the five most prevalent serovars from animal sources that fell into different priority serovar groups (DENT - *S.* Typhimurium; Marler – *S.* Anatum, *S.* Infantis and *S.* Saintpaul; NNDSS - *S.* Bovismorbificans) and for which there were enough animal and human isolates to make meaningful comparisons.

In general, these trees showed that some serovars of Australian *Salmonella* isolates clustered mostly by host (animal or human for *S.* Typhimurium, *S.* Infantis and *S*. Anatum), while other serovars did not cluster by host (*S.* Saintpaul and *S.* Bovismorbificans).

Salmonella Typhimurium isolates grouped into two major clusters, one cluster comprised mostly human isolates (Figure 3– yellow shaded cluster), while the other comprised mostly animal isolates (**Error! Reference source not found.** – green shaded cluster).

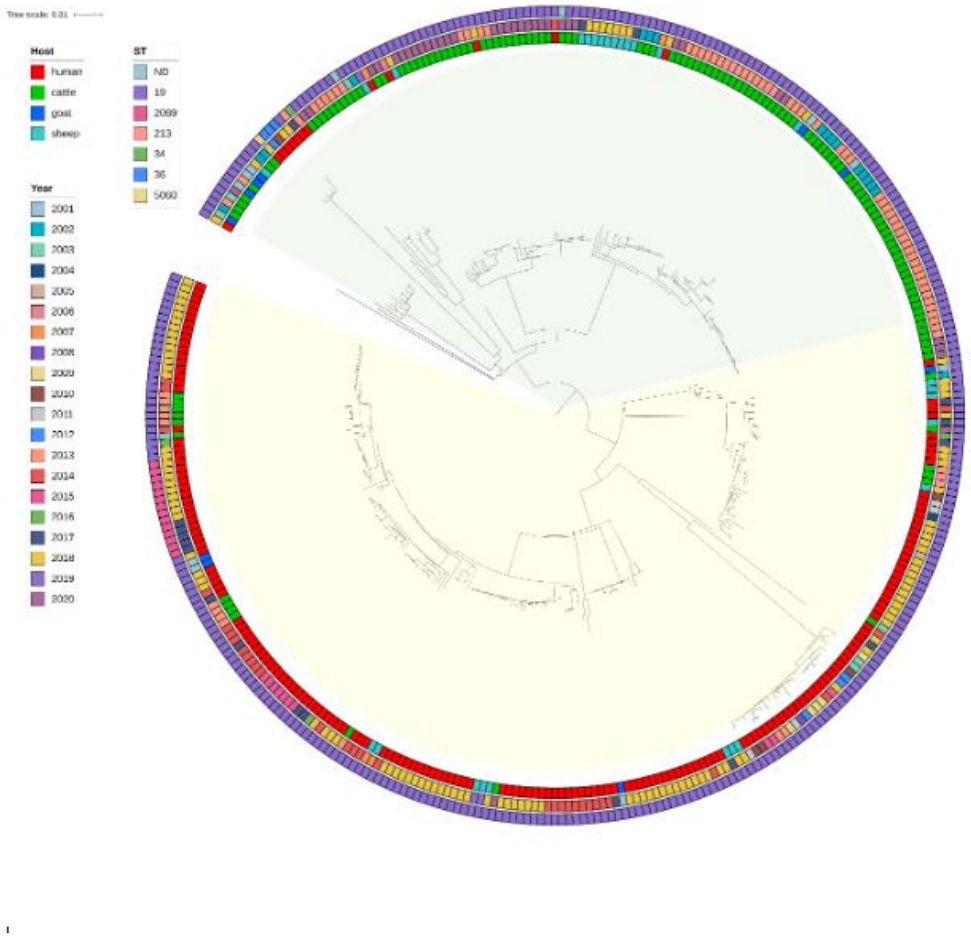

Figure 3 Phylogenetic tree of *Salmonella* Typhimurium isolates from animal and human sources in Australia. The inner-most coloured ring indicates the host of the isolates (red – human; green – cattle; dark blue – goat; teal blue – sheep), the middle ring represents the year of collection and the outer ring shows the Sequence Type (ST). The shaded areas (green and yellow) indicate two major clusters.

Salmonella Anatum isolates also largely grouped by host (Figure 4 – yellow or green shaded cluster) although most human isolates were from 2016 and were highly related which indicates they may have been associated with a human outbreak (although this information was not available as part of the metadata downloaded when accessing sequences). All *S*. Anatum belonged to a single ST type, ST64.

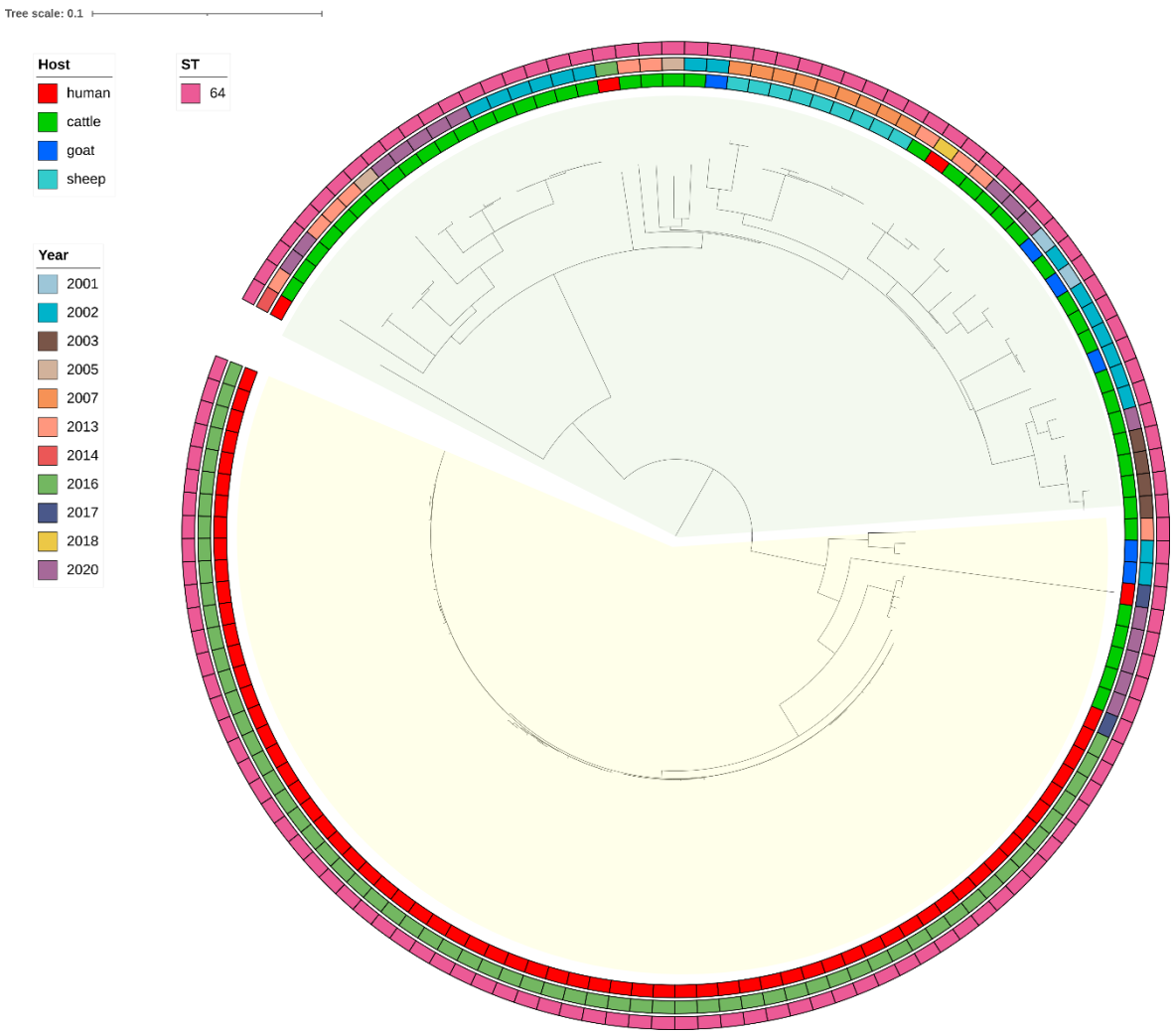

Figure 4. Phylogenetic tree of *Salmonella* Anatum isolates from animal and human sources in Australia. The inner-most coloured ring indicates the host of the isolates (red – human; green – cattle; dark blue – goat; teal blue – sheep), the middle ring represents the year of collection and the outer ring shows the Sequence Type (ST). The shaded areas (green and yellow) indicate two major clusters.

Salmonella Infantis was somewhat similar to *S.* Typhimurium where isolates grouped into two major clusters, one cluster comprised mostly human isolates (Figure 5– yellow shaded cluster), while the other comprised a mix with the majority of isolates coming from animal sources (Figure 5– green shaded cluster).

Figure 5. Phylogenetic tree of *Salmonella* Infantis isolates from animal and human sources in Australia. The inner-most coloured ring indicates the host of the isolates (red – human; green – cattle; dark blue – goat; teal blue – sheep), the middle ring represents the year of collection and the outer ring shows the Sequence Type (ST). The shaded areas (green and yellow) indicate two major clusters.

The majority of *Salmonella* Saintpaul formed branches that contained both human and animal isolates with most belonging to a single sequence type, ST50 (Figure 6).

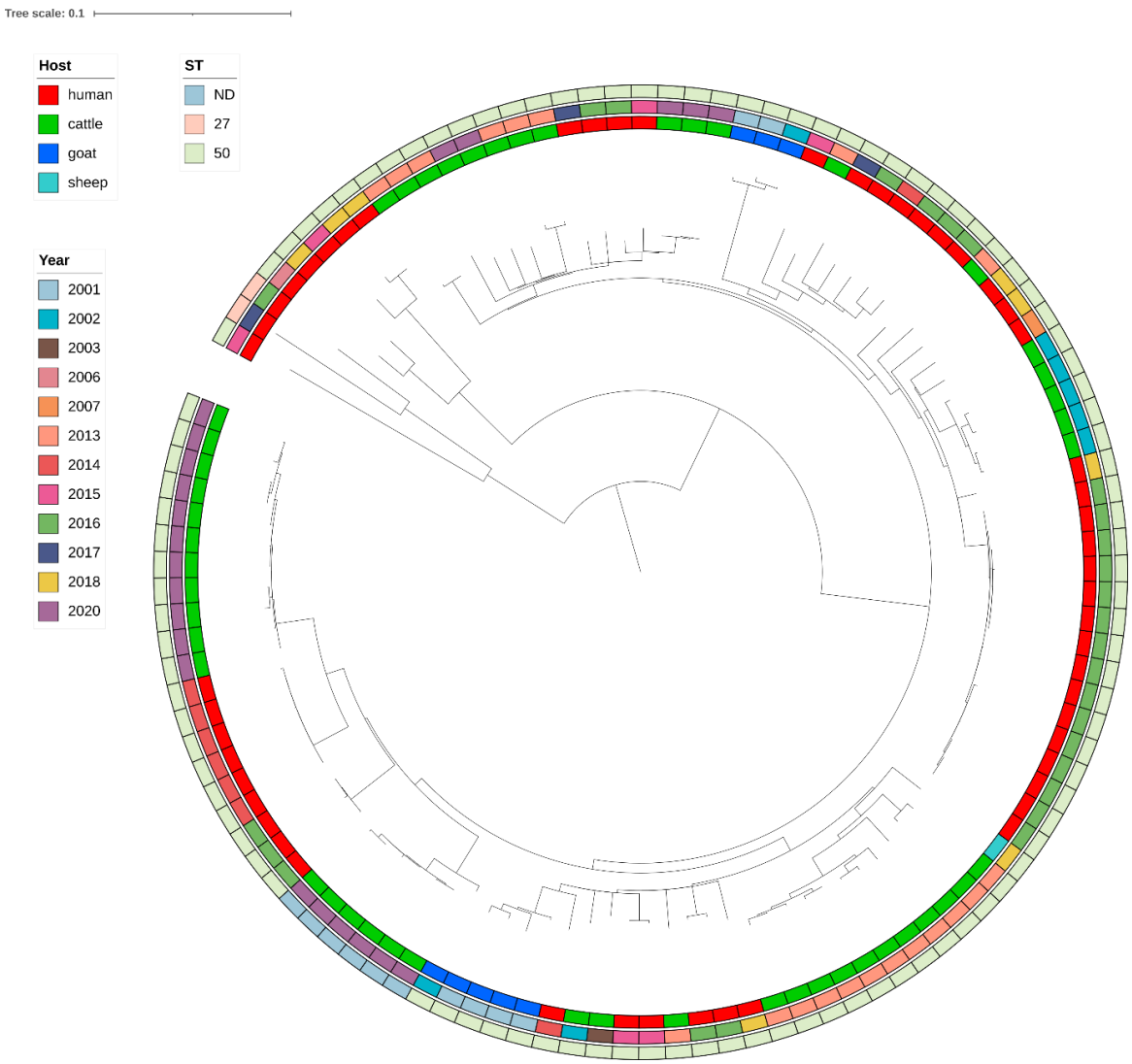

Figure 6. Phylogenetic tree of *Salmonella* Saintpaul isolates from animal and human sources in Australia. The inner-most coloured ring indicates the host of the isolates (red – human; green – cattle; dark blue – goat; teal blue – sheep), the middle ring represents the year of collection and the outer ring shows the Sequence Type (ST).

Salmonella Bovismorbificans clustered by ST, with most isolates belonging to ST377. Small distances between branches of the tree suggest a high degree of relatedness between isolates, and the observation of close relationships between isolates recovered from a range of different hosts and across many years suggests that *S*. Bovismorbificans isolates in Australia are highly related (clonal) and dominated by a single ST type (Figure 7).

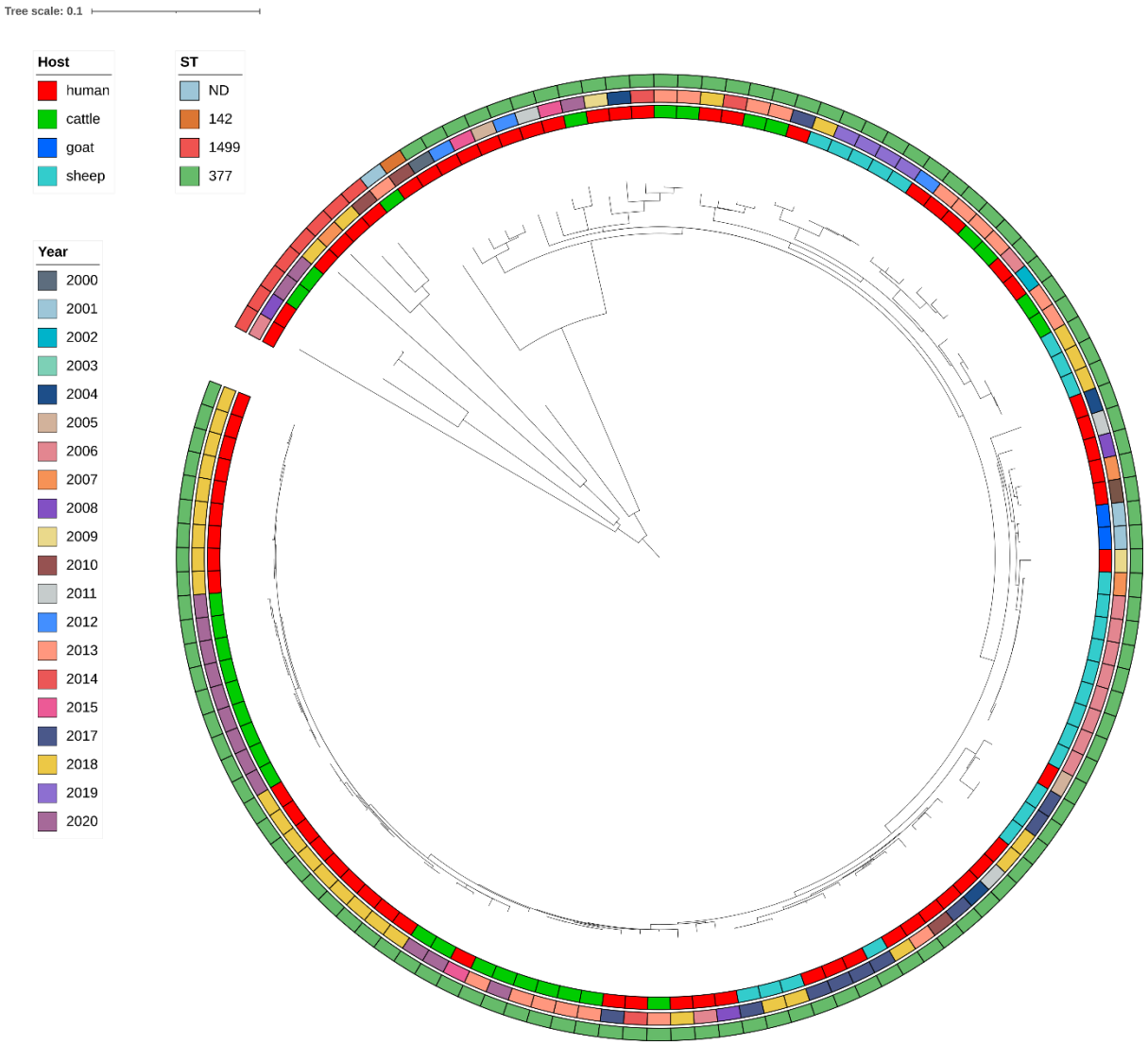

Figure 7. Phylogenetic tree of *Salmonella* Bovismorbificans isolates from animal and human sources in Australia. The inner-most coloured ring indicates the host of the isolates (red – human; green – cattle; dark blue – goat; teal blue – sheep), the middle ring represents the year of collection and the outer ring shows the Sequence Type (ST).

4.2.2 MLST comparisons

Multilocus sequence typing (MLST) is a molecular approach to characterising bacteria that has proven useful for studying the diversity and relatedness of pathogens in epidemiological investigation. The approach is less discriminatory than single nucleotide polymorphism (SNP tree) based methods of relationship assessment, but a major advantage is that isolates are assigned to an unambiguous sequence type, which is useful when comparing between studies.
In the current study, 49 different sequence types (ST) were identified among the 1081 isolates tested (Appendix 8.1). As expected, isolates broadly grouped into one or two sequence types based on serovar rather than source of isolation. Despite this, human and animal isolates within certain serovars that had common STs were shown to belong to different clades (branches) within SNP-based phylogenetic trees, suggesting a level of genetic diversity within a serovar that is not captured by the MLST method, due to its inherently lower discriminatory power. This highlights the value of exploring isolate relationships by different tools and the importance of choosing the most appropriate tool for the research question.

4.2.3 *Salmonella* pathogenicity islands

Salmonella pathogenicity islands (SPIs) are defined as large gene cassettes within the *Salmonella* chromosome that encode determinants responsible for establishing specific interactions within the host and are required for bacterial virulence in a given animal model (24).
While there are currently 24 SPIs identified only those noted in the SPI database used in this study are listed below with their primary function (Table 4).

Table 4. *Salmonella* pathogenicity islands (SPI) and associated functions, investigated in the current study.

SPI	Function
SPI-1	T3SS - Invasion, bacterial penetration of the epithelial cells (invA is in SPI1)
SPI-2	T3SS - Growth and survival in the host, intracellular survival and proliferation
SPI-3	Growth and survival in host,
SPI-4	T1SS – mediates toxin secretion
SPI-5	T3SS - Mediate inflammation and chloride secretion,
SPI-6	T6SS – encodes fimbrial systems
SPI-7	Exclusive to S. Typhi
SPI-8	T1SS, tRNA pheV
SPI-9	T1SS, adhesion
SPI-10	T1SS, Sef finbriae
SPI-11	Association with bacteriophage genomes and tRNA genes
SPI-12	tRNA
SPI-13	Role as yet unclear, not found in Typhi and Paratyphi
SPI-14	Role as yet unclear, not found in Typhi and Paratyphi

SPI-1 to 6, 9, 13 and 14 were present in most isolates, while SPI-8, 11 and 12 were absent from most (Table 5). While SPI-1 – SPI-5 are considered to be common to all serovars (25), past studies have demonstrated the absence of some of these SPIs across serovars (26). A study of human isolates recorded SPI-1 – SPI-5 and SPI-9 in all serovars, also noting that the detection of other SPIs was serovar specific (27).

In this study there was a noted difference (>10%) in the carriage of SPI-2 between isolates from human and animals across five serovars: Derby, Aberdeen, Heidelberg, Meunchen and Reading,

although there were limited numbers of isolates from host sources for these serovars to make firm conclusions. Nonetheless, SPI-2 is regarded a key pathogenicity island that's involved in systemic infections and intracellular pathogenesis through its actions inside the host cell (28), and further investigation on larger strain sets may be warranted.

While important for investigating pathogen potential, SPIs have also been used to identify host adapted *Salmonella* serovars. For instance, *S*. Derby has adapted to both porcine and poultry hosts with different genomic clones circulating in these hosts. The differences between them lies in the SPIs and other genomic characteristics (26). Specifically, SPI-2 was noted to carry a variant in the sequence of the STM1546 gene involved in protecting DNA from damage. Investigation of SPI variants were beyond the scope of the current study, but it may be an area for further investigation.

Except for a small number of *S*. Bovismorbificans and *S*. Anatum, isolates in this study did not contain SPI-12. Some *S*. Kentucky chicken derived *Salmonella* isolates have been found to lack some SPI-12 related genes which is suggested as a reason why Kentucky has a lower rate of disease in humans (29). It is most likely the use of different bioinformatic pipelines and different SPI databases that differences exist between published studies and the current study (30), though further work is required to confirm this.

In the current study, isolates belonging to *S*. Agona were the only isolates to possess SPI-8, while isolates belonging to *S*. Agona, Derby and Mbandaka either lacked or had lower carriage rates of SPI-6, 13 and 14. In agreement with this, past studies have noted the lack the carriage of SPI-13 and 14 in human isolates of Derby, Agona and Kentucky (27) while SPI-8 was observed only in Agona (27). Likewise, other studies have highlighted variability in the carriage of SPI-13 and 14 across serovars (31) with Agona, Derby, Mbandaka amongst those shown not to carry SPI-13 and 14 (31). The T6SS encoded in SPI-6 has been found to be widely distributed within *Salmonella enterica* serovars (32) with some serovars carrying multiple T6SS and some none at all. However, the T6SS is not essential for virulence.

S. Enteritidis carried SPI-11 at 18.8% in human derived isolates. Luo et al, 2021 (33) noted differences in the carriage of SPIs within *S*. Enteritidis with some isolates carrying SPIs with partial absence of some genes. SPI-11 was found to be intact only within a single sequence type. Other STs had four or five genes missing from SPI-11. Within the Australian context it has been noted that different clades of *S.* Enteritidis carry varying virulence factors with complete SPI-6 and 19 found in clades A and C, largely from locally acquired infections, while also noting that clade B grouped with global isolates (34).

Table 5. Percent of isolates that possess *Salmonella* Pathogenicity Islands (SPI)

Predicted Serovar	Host	Count	SPI-1	SPI-2	SPI-3	SPI-4	SPI-5	SPI-6	SPI-8	SPI-9	SPI-11	SPI-12	SPI-13	SPI-14	SESS LEE	SGI1	HPI	CS54 island
Aberdeen	animal	5	100	80	100	100	100	100	0	100	0	0	100	100	100	100	0	100
Aberdeen	human	1	100	100	100	100	100	100	0	100	0	0	100	100	100	100	0	100
Agona	animal	20	100	100	100	100	0	75	100	100	0	0	85	85	100	100	0	100
Agona	human	21	100	95.2	100	100	0	0	90.5	100	0	0	0	0	100	100	0	100
Anatum	animal	60	100	93.3	100	100	100	100	0	98.3	0	1.7	100	100	100	100	0	100
Anatum	human	70	100	100	100	100	100	100	0	100	0	0	100	100	100	100	0	100
Bovismorbificans	animal	56	100	92.9	100	100	98.2	100	0	98.2	0	13	100	100	98.2	100	1.8	100
Bovismorbificans	human	66	100	98.5	100	100	100	100	0	100	0	3.0	100	100	100	100	0	100
Chester	animal	14	100	100	100	100	100	100	0	100	0	0	100	100	100	100	0	100
Chester	human	15	100	100	100	100	100	100	0	100	0	0	100	100	100	100	0	100
Derby	animal	1	100	0	100	100	0	0	0	100	0	0	100	100	100	100	0	100
Derby	human	3	100	100	100	100	0	0	0	100	0	0	0	0	100	100	0	100
Dublin	animal	3	100	100	100	100	100	100	0	100	0	0	100	100	100	100	0	100
Dublin	human	17	100	100	100	100	100	100	0	100	0	0	100	100	100	100	0	100
Gallinarum or Enteritidis	animal	1	100	100	100	100	100	100	0	100	0	0	100	100	100	100	0	100
Enteritidis	human	16	100	100	100	100	100	100	0	100	18.8	0	100	100	100	100	0	100
Heidelberg	animal	8	100	87.5	100	100	100	100	0	100	0	0	100	100	100	100	0	100
Heidelberg	human	5	100	100	100	100	100	100	0	100	0	0	100	100	100	100	0	100
Hvittingfoss	animal	2	100	100	100	100	100	100	0	100	0	0	100	100	100	100	0	100
Hvittingfoss	human	3	100	100	100	100	100	100	0	100	0	0	100	100	100	100	0	100
Infantis	animal	26	100	92.3	100	100	96.2	100	0	96.2	0	0	100	100	100	100	3.9	100
Infantis	human	37	100	100	100	100	100	100	0	100	0	0	100	100	100	100	0	100
Kottbus	animal	3	100	100	100	100	100	100	0	100	0	0	100	100	100	100	0	100
Litchfield	animal	1	100	100	100	100	100	100	0	100	0	0	100	100	100	100	0	100
Litchfield	human	1	100	100	100	100	100	100	0	100	0	0	100	100	100	100	0	100
Mbandaka	animal	17	100	100	100	100	0	52.9	0	100	0	0	70.6	58.8	100	100	0	100
Mbandaka	human	4	100	100	100	100	0	0	0	100	0	0	0	0	100	100	0	100
Montevideo	animal	16	100	100	100	100	100	100	0	100	0	0	100	100	100	100	0	100
Montevideo	human	2	100	100	100	100	100	100	0	100	0	0	100	100	100	100	0	100
Muenchen	animal	25	100	96	100	100	100	100	0	100	0	0	100	100	100	100	0	100
Muenchen	human	5	100	80	100	100	100	100	0	100	0	0	100	100	100	100	0	100
Newport	animal	3	100	100	100	100	100	100	0	100	0	0	100	100	100	100	0	100
Newport	human	19	100	100	100	100	100	100	0	100	0	0	100	100	100	100	0	100
Poona	animal	3	100	100	100	100	100	100	0	100	0	0	100	100	100	100	0	100
Poona	human	4	100	100	100	100	100	100	0	100	0	0	100	100	100	100	0	100
Reading	animal	21	100	95.2	100	100	100	100	0	100	0	0	100	100	100	100	0	100
Reading	human	3	100	66.7	100	100	100	100	0	100	0	0	100	100	100	100	0	100
Saintpaul	animal	58	100	100	100	100	100	100	0	100	0	0	100	100	100	100	0	100
Saintpaul	human	53	100	98.11	100	100	100	100	0	100	0	0	100	100	100	100	0	100
Singapore	animal	1	100	100	100	100	100	100	0	100	0	0	100	100	100	100	0	100
Singapore	human	1	100	100	100	100	100	100	0	100	0	0	100	100	100	100	0	100
Thompson	human	8	100	100	100	100	100	100	0	100	0	0	100	100	100	100	0	100
Typhimurium	animal	164	100	98.8	100	100	98.8	100	0	99.4	0	0	100	100	100	100	0	100
Typhimurium	human	187	100	98.9	100	100	99.5	100	0	100	0	0	100	100	100	100	0	100
Virchow	animal	15	100	93.3	100	100	100	100	0	100	0	0	100	100	100	100	0	100
Virchow	human	16	100	100	100	100	100	100	0	100	0	0	100	100	100	100	0	100
Wangata	animal	1	100	100	100	100	100	100	0	100	0	0	100	100	100	100	0	100

4.2.4 Virulence gene comparisons

All *Salmonella* isolate sequences were assessed against a database of virulence genes from 32 genera of medical importance. Of the ~9000 genes present in the database, 136 of these were found in the genomes of Australian *Salmonella* (Table 6). In general, isolates from within a serovar from different sources had similar virulence gene profiles. For serovars belonging to DENT, no host-based comparisons were able to be conducted for *S.* Enteritidis, *S.* Newport and *S.* Dublin as there were too few animal isolates to draw any conclusions.

Comparison between animal and human isolates within a serovar.

S. Typhimurium isolates were compared across different sources with a higher percentage of human and sheep isolates (≥89%) carrying *pef*, *rck* and *spv* genes than isolates from cattle or goats (≤50%). These genes are associated with a plasmid, pSLT, found in *S.* Typhimurium that confers pathogenicity (35). The *Salmonella* plasmid virulence (*spv* operon and resistance to complement killing (*rck*) is important in survival within the host, while plasmid encoded fimbriae (*pef* operon) is thought to aid in adhesion of *Salmonella* to host epithelial cells.

Salmonella Anatum isolates from animals contained a greater diversity of virulence genes (*gyrA*, *lpfA*/*B*/*C*/*D*/*E*, *shdA*, *sseKI*) than those from humans where these genes were not detected, although ≤33% of isolates from any one animal source carried these genes. The gene *sspH1* (associated with type three secretion systems) (36) was found in most cattle isolates (53%) and in 1% of human isolates of *S.* Anatum. Miao *et al* (37) suggest that SspH1 and SspH2 play a role in bovine virulence, with *S*. Typhimurium showing increased virulence in calves when *sspH1* and *sspH2* were present and lower virulence when these genes were deleted in the same strain of *S*. Typhimurium.

The *S.* Bovismorbificans isolates virulence gene profiles were similar between animal and human isolates, with the exception of *shdA* (a gene associated with prolonged faecal shedding of *Salmonella* in animals) (38), where between 50-90% of animal isolates carried this gene while it was found in only 2% of human isolates.

The most variability in virulence gene profiles based on host were found in *S.* Infantis isolates, although only a small number of sheep (6) and goat (5) isolates were available for comparison. In particular, isolates from human (41%) and sheep (17%) carried *irp1/2* (encoding iron regulatory proteins) (39) and *fyuA* and the *ybt* operon genes (40) though these were not found in the cattle or goat isolates. *FyuA* and *ybt* are associated with the Yersinia high pathogenicity island and play a role in iron acquisition and have been reported in *S. Heidelberg* (40).

For *S.* Saintpaul isolates, three genes (*sodC1*, *sseI*/*srfH*, *grvA*) were approximately twice as likely to occur among animals than humans, while two genes, *gogB* and *spH1*, were approximately 5 and 2 times respectively more likely to occur in human isolates than animals.

Comparison between serovars

Several genes were shown to be highly associated with a single or multiple serovars. For instance, the *entA* gene was present in 97%-100% of *S.* Anatum, *S.* Bovismorbificans, *S.* Saintpaul and *S.* Typhimurium, but absent from all *S.* Infantis isolates, regardless of host. Likewise, the *faeC*, *D* and *E* genes were present in greater than 98% of *S.* Anatum and Saintpaul, and largely absent from S. Bovismorbificans and *S*. Typhimurium. Multiple other examples of serovar specific genes are shown in Table 6. Such genes may have an influence on the colonisation, survival or shedding in animals and the pathogenic potential in humans, and a deeper exploration of these may be useful for understanding serovar differences in disease potential.

Table 6. Heat map of *Salmonella* isolates from various hosts (% of isolates by host)

Gene	*S.* Anatum				*S.* Bovismorbificans				*S.* Infantis				*S.* Saintpaul				*S.* Typhimurium			
	Cattle n=45	Goat n=6	Sheep n=9	Human n=70	Cattle n=30	Goat n=2	Sheep n=24	Human n=66	Cattle n=15	Goat n=5	Sheep n=6	Human n=37	Cattle n=49	Goat n=8	Sheep n=1	Human n=53	Cattle n=127	Goat n=10	Sheep n=27	Human n=187
astA	2	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	1	0	0	0
avrA	100	100	100	100	97	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
cdtB	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
csgA	100	100	100	100	100	100	100	100	100	100	83	100	100	100	100	100	100	100	100	100
csgB	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	99	100	100	100
csgC	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
csgD	100	100	100	100	97	100	100	100	100	100	83	100	100	100	100	100	100	100	100	100
csgE	100	100	100	100	97	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
csgF	100	100	100	100	97	100	100	100	100	100	100	100	100	100	100	100	99	100	100	100
csgG	100	100	100	100	97	100	100	100	100	100	83	100	100	100	100	100	100	100	100	100
entA	100	100	100	100	97	100	100	100	0	0	0	0	100	100	100	100	99	100	100	100
entB	100	100	100	100	97	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
faeC	100	100	100	100	7	0	0	0	7	0	0	0	100	100	100	100	3	0	0	0
faeD	100	100	100	99	0	0	0	0	0	0	0	41	100	100	100	100	0	0	0	0
faeE	100	100	100	99	3	0	0	0	13	0	0	41	98	100	100	100	1	0	0	0
faeF	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
faeH	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
fepC	100	100	100	100	100	100	100	100	100	100	83	100	100	100	100	100	100	100	100	100
fepG	100	100	100	100	97	100	100	100	100	100	83	100	100	100	100	100	99	100	100	100
fimC	100	100	100	100	97	100	100	100	100	100	83	100	100	100	100	100	100	100	100	100
fimD	100	100	100	100	97	100	100	100	100	100	83	100	100	100	100	100	100	100	100	100
fimF	100	100	100	100	97	100	100	100	100	100	100	100	100	100	100	100	99	100	100	100
fimH	98	100	100	100	97	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
fimI	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	99	100	100	100
fyuA	0	0	0	0	0	0	0	0	0	0	17	41	0	0	0	0	0	0	0	0
gogB	0	0	0	0	60	0	29	59	0	0	17	0	6	0	0	34	94	100	100	97
grvA	7	17	11	0	7	50	13	5	13	0	33	0	61	63	100	30	96	100	100	98
hlyB	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	3
hlyD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	3
invA	100	100	100	100	97	100	100	100	100	100	83	100	100	100	100	100	99	100	100	100
invB	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
invC	100	100	100	100	97	100	100	100	100	100	83	100	100	100	100	100	100	100	100	100

Gene	*S.* Anatum				*S.* Bovismorbificans				*S.* Infantis				*S.* Saintpaul				*S.* Typhimurium			
	Cattle n=45	Goat n=6	Sheep n=9	Human n=70	Cattle n=30	Goat n=2	Sheep n=24	Human n=66	Cattle n=15	Goat n=5	Sheep n=6	Human n=37	Cattle n=49	Goat n=8	Sheep n=1	Human n=53	Cattle n=127	Goat n=10	Sheep n=27	Human n=187
invE	100	100	100	100	100	100	100	100	100	100	83	100	100	100	100	100	100	100	100	100
invF	100	100	100	100	100	100	100	100	100	100	83	100	100	100	100	100	100	100	100	100
invG	100	100	100	100	97	100	100	100	100	100	83	100	100	100	100	100	99	100	100	100
invH	100	100	100	100	97	100	100	100	100	100	83	100	100	100	100	100	100	100	100	100
invI	100	100	100	100	97	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
invJ	100	100	100	100	100	100	100	100	100	100	83	100	100	100	100	100	100	100	100	100
irp1	0	0	0	0	0	0	0	0	0	0	17	41	0	0	0	0	0	0	0	0
irp2	0	0	0	0	0	0	0	0	0	0	17	41	0	0	0	0	0	0	0	0
lpfA	18	33	0	0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
lpfB	13	17	11	0	97	100	100	100	100	100	100	100	100	100	100	100	99	100	100	100
lpfC	2	0	0	0	97	100	100	100	100	100	83	100	100	100	100	100	100	100	100	100
lpfD	7	0	0	0	97	100	100	100	100	100	83	100	100	100	100	100	100	100	100	100
lpfE	20	33	0	0	97	100	100	100	100	100	83	100	100	100	100	100	100	100	100	100
mgtB	100	100	100	100	97	100	100	100	100	100	83	100	100	100	100	100	99	100	100	100
mgtC	100	100	100	100	100	100	100	100	100	100	83	100	100	100	100	100	100	100	100	100
mig-14	100	100	100	100	97	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
misL	89	100	100	100	97	100	100	100	100	100	83	100	100	100	100	100	99	100	100	100
ompA	100	100	100	100	97	100	100	100	100	100	83	100	100	100	100	100	99	100	100	100
orgA	100	100	100	100	100	100	100	100	100	100	83	100	100	100	100	100	100	100	100	100
orgB	100	100	100	100	97	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
orgC	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
pefA	0	0	0	0	90	100	100	92	0	0	0	0	0	0	0	0	41	50	89	96
pefB	2	0	0	0	90	100	100	92	7	0	17	0	2	0	0	0	41	50	89	96
pefC	0	0	0	0	90	100	100	92	0	0	0	0	0	0	0	0	39	50	89	96
pefD	0	0	0	0	90	100	100	92	0	0	0	0	0	0	0	0	39	50	89	96
pipB	100	100	100	100	97	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
pipB2	98	100	100	100	97	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
prgH	100	100	100	100	97	100	100	100	100	100	83	100	100	100	100	100	99	100	100	100
prgI	100	100	100	100	100	100	100	100	100	100	83	100	100	100	100	100	100	100	100	100
prgJ	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Gene	*S.* Anatum				*S.* Bovismorbificans				*S.* Infantis				*S.* Saintpaul				*S.* Typhimurium			
	Cattle n=45	Goat n=6	Sheep n=9	Human n=70	Cattle n=30	Goat n=2	Sheep n=24	Human n=66	Cattle n=15	Goat n=5	Sheep n=6	Human n=37	Cattle n=49	Goat n=8	Sheep n=1	Human n=53	Cattle n=127	Goat n=10	Sheep n=27	Human n=187
prgK	100	100	100	100	100	100	100	100	100	100	83	100	100	100	100	100	100	100	100	100
ratB	2	0	0	0	97	100	100	100	100	100	100	100	100	100	100	100	99	100	100	100
rck	0	0	0	0	90	100	100	92	0	0	0	0	0	0	0	0	39	50	89	96
shdA	20	17	11	0	67	50	92	2	100	100	83	86	2	0	0	0	2	0	4	0
sicA	100	100	100	100	100	100	100	100	100	100	83	100	100	100	100	100	100	100	100	100
sicP	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
sifA	100	100	100	100	97	100	100	100	100	100	100	100	100	100	100	100	99	100	100	100
sifB	98	100	100	100	100	100	100	100	100	100	83	100	100	100	100	100	100	100	100	100
sinH	100	100	100	100	97	100	100	100	100	100	83	100	100	100	100	100	99	100	100	100
sipA/sspA	100	100	100	100	93	100	100	100	100	100	83	100	100	100	100	100	100	100	100	100
sipB/sspB	100	100	100	100	97	100	100	100	100	100	83	100	100	100	100	100	100	100	100	100
sipC/sspC	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
sipD	100	100	100	100	97	100	100	100	100	100	83	100	100	100	100	100	100	100	100	100
slrP	100	100	100	100	100	100	100	100	100	100	83	100	100	100	100	100	99	100	100	100
sodCI	4	0	0	0	97	100	100	97	20	0	50	0	61	50	100	34	100	100	100	99
sopA	100	100	100	100	97	100	100	100	100	100	83	100	100	100	100	100	99	90	100	100
sopB/sigD	100	100	100	100	97	100	100	100	100	100	83	100	100	100	100	100	98	100	100	100
sopD	98	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
sopD2	100	100	100	100	97	100	100	100	100	100	83	100	100	100	100	100	99	100	100	100
sopE2	100	100	100	100	97	100	100	100	100	100	83	100	100	100	100	100	100	100	100	100
spaO	100	100	100	100	97	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
spaP	100	100	100	100	100	100	100	100	100	100	83	100	100	100	100	100	99	100	100	100
spaQ	100	100	100	100	97	100	100	100	100	100	83	100	100	100	100	100	100	100	100	100
spaR	100	100	100	100	97	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
spaS	100	100	100	100	100	100	100	100	100	100	83	100	100	100	100	100	100	100	100	100
spiC/ssaB	100	100	100	100	97	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
sptP	100	100	100	100	97	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
spvB	0	0	0	0	90	100	100	92	0	0	0	0	0	0	0	0	39	50	89	96
spvC	0	0	0	0	90	100	100	92	0	0	0	0	0	0	0	0	40	50	89	96
spvR	0	0	0	0	90	100	100	92	0	0	0	0	0	0	0	0	39	50	89	96

Gene	*S.* Anatum				*S.* Bovismorbificans				*S.* Infantis				*S.* Saintpaul				*S.* Typhimurium			
	Cattle n=45	Goat n=6	Sheep n=9	Human n=70	Cattle n=30	Goat n=2	Sheep n=24	Human n=66	Cattle n=15	Goat n=5	Sheep n=6	Human n=37	Cattle n=49	Goat n=8	Sheep n=1	Human n=53	Cattle n=127	Goat n=10	Sheep n=27	Human n=187
ssaC	100	100	100	100	97	100	100	100	100	100	83	100	100	100	100	100	99	100	100	100
ssaD	100	100	100	100	97	100	100	100	100	100	83	100	100	100	100	100	100	100	100	100
ssaE	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
ssaG	100	100	100	100	97	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
ssaH	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
ssaI	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
ssaJ	100	100	100	100	97	100	100	100	100	100	83	100	100	100	100	100	100	100	100	100
ssaK	100	100	100	100	100	100	100	100	100	100	83	100	100	100	100	100	100	100	100	100
ssaL	100	100	100	100	97	100	100	100	100	100	83	100	100	100	100	100	100	100	100	100
ssaM	100	100	100	100	97	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
ssaN	100	100	100	100	100	100	100	100	100	100	83	100	100	100	100	100	99	100	100	100
ssaO	100	100	100	100	97	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
ssaP	100	100	100	100	97	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
ssaQ	100	100	100	100	97	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
ssaR	100	100	100	100	97	100	100	100	100	100	100	100	100	100	100	100	99	100	100	100
ssaS	100	100	100	100	97	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
ssaT	100	100	100	100	97	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
ssaU	100	100	100	100	97	100	100	100	100	100	83	100	100	100	100	100	99	100	100	100
ssaV	100	100	100	100	97	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
sscA	100	100	100	100	97	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
sscB	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
sseA	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
sseB	100	100	100	100	100	100	100	100	100	100	83	100	100	100	100	100	100	100	100	100
sseC	100	100	100	100	100	100	100	100	100	100	83	100	100	100	100	100	100	100	100	100
sseD	100	100	100	100	97	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
sseE	100	100	100	100	97	100	100	98	100	100	100	100	100	100	100	100	99	100	100	100
sseF	100	100	100	100	100	100	100	100	100	100	83	100	100	100	100	100	100	100	100	100
sseG	100	100	100	100	97	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
sseI/srfH	0	0	0	0	97	100	100	97	0	0	0	0	45	50	100	17	99	100	100	99
sseJ	100	100	100	100	97	100	100	100	100	100	83	100	100	100	100	100	99	100	100	100

Gene	*S.* Anatum				*S.* Bovismorbificans				*S.* Infantis				*S.* Saintpaul				*S.* Typhimurium			
	Cattle n=45	Goat n=6	Sheep n=9	Human n=70	Cattle n=30	Goat n=2	Sheep n=24	Human n=66	Cattle n=15	Goat n=5	Sheep n=6	Human n=37	Cattle n=49	Goat n=8	Sheep n=1	Human n=53	Cattle n=127	Goat n=10	Sheep n=27	Human n=187
sseK1	9	17	0	0	97	100	100	100	100	100	100	100	2	0	0	0	100	100	100	100
sseK2	98	100	100	100	97	100	100	98	100	100	100	100	100	100	100	100	100	100	100	100
sseL	100	100	100	100	97	100	100	100	100	100	100	97	100	100	100	100	99	100	100	99
sspH1	53	0	0	1	0	0	0	0	0	0	0	0	16	13	0	34	0	0	0	0
sspH2	100	100	100	96	97	100	100	89	100	100	83	81	100	100	100	96	99	100	100	91
steA	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
steB	100	100	100	100	97	100	100	100	100	100	100	100	100	100	100	100	99	100	100	100
steC	100	100	100	100	97	100	100	100	100	100	83	100	100	100	100	100	99	100	100	100
tcpC	100	100	100	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ybtA	0	0	0	0	0	0	0	0	0	0	17	41	0	0	0	0	0	0	0	0
ybtE	0	0	0	0	0	0	0	0	0	0	17	41	0	0	0	0	0	0	0	0
ybtP	0	0	0	0	0	0	0	0	0	0	17	41	0	0	0	0	0	0	0	0
ybtQ	0	0	0	0	0	0	0	0	0	0	17	41	0	0	0	0	0	0	0	0
ybtS	0	0	0	0	0	0	0	0	0	0	17	41	0	0	0	0	0	0	0	0
ybtT	0	0	0	0	0	0	0	0	0	0	17	41	0	0	0	0	0	0	0	0
ybtU	0	0	0	0	0	0	0	0	0	0	17	41	0	0	0	0	0	0	0	0
ybtX	0	0	0	0	0	0	0	0	0	0	17	41	0	0	0	0	0	0	0	0

4.2.5 Resistome analysis

A resistome analysis was conducted to look for the presence of genes known to be associated with antimicrobial resistance (AMR) in *Salmonella* from different hosts (Table 7). AMR genes were detected in very low percentages of the *Salmonella* isolates with the exception of *aac(6')-Iaa*, which was found in nearly all of the isolates. This gene is a chromosomally encoded aminoglycoside acetyltransferase found in *Salmonella*. For the remaining AMR genes, many were absent or detected only in a low percentage of isolates. Specifically, most genes were entirely absent from animals, and while 60 of the 71 AMR genes were detected in at least 1 human isolate, the percent of isolates carrying each AMR gene was low. Animal isolates of *Salmonella* contained less diversity of AMR genes than those from human sources with 13 different AMR genes found in cattle isolates, 11 in goat isolates and only 2 different AMR genes found in isolates from sheep. Goat and sheep isolates were more likely to carry *fosA7* (21.74% and 12.64% respectively) than human (5.21%) or cattle (1.79%) isolates. *FosA7*, along with *fosA3*, encode for Fosfomycin resistance and are often found in *Salmonella*, including those isolated from animal-based foods (41). The relatively low presence of antimicrobial resistance genes among Australian animal isolates aligns with past studies that show a low prevalence of AMR in Australian red meat animals.

Table 7. Heat map of *Salmonella* isolates from various hosts (% of isolates by host) and the presence of antimicrobial resistance genes (green indicates the gene was not present in any isolates). Colours indicate the percent of isolates from the host category which contained that virulence gene: red – gene was detected in >99% of isolates, orange - 20-99% and yellow < 20%.

Resistance Gene	Host			
	Cattle n=391	Goat n=46	Sheep n=87	Human n=557
ARR-3	0.0	0.0	0.0	0.7
aac(3)-IId	0.0	0.0	0.0	1.4
aac(3)-IVa	0.0	0.0	0.0	2.3
aac(6')-IIc	0.0	0.0	0.0	0.2
aac(6')-Iaa	100.0	100.0	100.0	99.8
aac(6')-Ib-cr	0.0	0.0	0.0	0.2
aac(6')-aph(2'')	0.0	0.0	0.0	0.0
aadA16	0.0	0.0	0.0	0.2
aadA2	0.0	0.0	0.0	1.8
aadA7	0.0	0.0	0.0	0.2
ant(2'')-Ia	0.0	0.0	0.0	0.5
ant(3'')-Ia	0.8	2.2	0.0	3.4
ant(6)-Ia	0.0	0.0	0.0	0.0
aph(3'')-Ib	0.8	2.2	0.0	3.8
aph(3')-III	0.0	0.0	0.0	0.0
aph(3')-Ia	0.0	0.0	0.0	2.7
aph(4)-Ia	0.0	0.0	0.0	2.3
aph(6)-Id	0.8	2.2	0.0	4.1
blaCARB-2	0.0	0.0	0.0	0.2
blaCMY-2	1.3	0.0	0.0	3.1
blaCTX-M-14	0.0	0.0	0.0	0.5
blaCTX-M-3	0.0	0.0	0.0	0.2

Resistance Gene	Host			
	Cattle n=391	Goat n=46	Sheep n=87	Human n=557
dfrA1	0.8	2.2	0.0	0.0
dfrA12	0.0	0.0	0.0	0.9
dfrA14	0.0	0.0	0.0	2.7
dfrA23	0.0	0.0	0.0	0.2
dfrA27	0.0	0.0	0.0	0.2
dfrA5	0.3	0.0	0.0	2.0
dfrB4	0.0	0.0	0.0	0.2
dfrG	0.0	0.0	0.0	0.0
ere(A)	0.0	0.0	0.0	0.2
erm(B)	0.0	0.0	0.0	0.0
floR	0.0	0.0	0.0	4.3
fosA3	0.0	0.0	0.0	0.9
fosA7	1.8	21.7	12.6	5.2
lnu(F)	0.0	0.0	0.0	0.2
mcr-9	0.0	0.0	0.0	0.5
mecA	0.0	0.0	0.0	0.0
mph(A)	0.0	0.0	0.0	0.7
mph(B)	0.8	2.2	0.0	0.0
oqxA	0.0	0.0	0.0	0.2
oqxB	0.0	0.0	0.0	0.2
qepA2	0.0	0.0	0.0	0.2
qnrA1	0.0	0.0	0.0	0.2

blaCTX-M-55	0.0	0.0	0.0	0.5	*qnrB19*	0.0	0.0	0.0	0.2
blaCTX-M-65	0.0	0.0	0.0	2.3	*qnrB4*	0.0	0.0	0.0	0.2
blaCTX-M-9	0.0	0.0	0.0	0.5	*qnrB6*	0.0	0.0	0.0	0.2
blaDHA-1	0.0	0.0	0.0	0.2	*qnrS1*	0.0	0.0	0.0	3.9
blaOXA-10	0.0	0.0	0.0	0.2	*sul1*	0.8	2.2	0.0	4.5
blaTEM-106	0.0	0.0	0.0	0.4	*sul2*	0.8	2.2	0.0	3.9
blaTEM-1A	0.0	0.0	0.0	0.2	*sul3*	0.0	0.0	0.0	1.6
blaTEM-1B	1.0	2.2	0.0	6.3	*tet(40)*	0.0	0.0	0.0	0.0
blaTEM-1D	0.0	0.0	0.0	0.2	*tet(A)*	1.0	2.2	0.0	8.4
blaZ	0.0	0.0	0.0	0.0	*tet(B)*	0.0	0.0	0.0	0.9
catA1	0.0	0.0	0.0	0.4	*tet(D)*	0.0	0.0	0.0	0.2
catA2	0.0	0.0	0.0	0.2	*tet(G)*	0.0	0.0	0.0	0.2
cmlA1	0.0	0.0	0.0	0.7	*tet(M)*	0.0	0.0	0.0	1.1
					tet(O)	0.0	0.0	0.0	0.0

5. Conclusion

It is mandatory for Australian export establishments to meet the food safety regulatory requirements of destination markets. To do this effectively, the industry should keep abreast of international developments in risk-based regulations that have the potential to impact market access and trade.

The USDA has recently accelerated efforts to meet a self-imposed 2030 target of reducing *Salmonella* infections associated with FSIS-regulated products by 25%. In a major step towards achieving this goal, the USDA have announced their intent to declare *Salmonella* an adulterant of certain poultry products. In a similar vein, the Marler Clark Food Safety Law Firm, has petitioned the FSIS to declare 31 serovars of *Salmonella* adulterants of meat and poultry products in the US and the USMARC, a branch of the USDA, have begun developing new diagnostic tools for the detection and quantitation of high-risk *Salmonella*. As a major exporter of beef to the US, it is important for the Australian industry to understand the domestic significance of these developments.

While there is no indication of intent by the FSIS to regulate *Salmonella* in beef, this project set out to perform an initial genomics assessment of Australian *Salmonella* to improve our understanding of risk. The project aimed to assess previously isolated *Salmonella* from beef, sheep and goat sources to determine (i) if the major disease-causing serovars identified align with the highly pathogenic *Salmonella* or DENT serovars and to (ii) determine if *Salmonella* from red meat supply chains align with Australian human clinical cases. The study analysed a large representative set of Australian *Salmonella*, and findings suggest that except for *S*. Typhimurium, very few of the HPS belonging to DENT serovars were observed. Relationship assessments showed that some serovars of Australian *Salmonella* clustered mostly by host (animal or human), suggesting that red meat animals/products may not be a major contributor to the human disease burden of these serovars in Australia. For other serovars, no obvious distinction between animal and human isolates was observed.

The genomics data collected in this study represents most of the *Salmonella* strains isolated from the Australian red meat industry by CSIRO between 2001 and 2019. The genomics analytics capability and genomics data can be accessed by industry to rapidly assess Australian *Salmonella* against future molecular based systems for defining highly pathogenic *Salmonella*. This resource will serve as an asset in the risk assessment and risk management of *Salmonella* in the Australian and

global supply chain and acts as an important resource to manage potential future market access risks for the red meat industry.

5.1 Key findings

Relationship assessment

- Except for *S.* Typhimurium, few *Salmonella* from Australian red meat sources belonged to the major DENT (Dublin, Enteritidis, Newport, Typhimurium, I 4,[5],12:i:-) categories.
- Phylogenetic trees showed that some serovars of Australian *Salmonella* isolates clustered mostly by host (animal or human for *S.* Typhimurium, *S.* Infantis, *S*. Anatum), while no obvious distinction between animal and human isolates was observed for other serovars (*S.* Saintpaul and *S.* Bovismorbificans).
- Isolates largely grouped into MLST types based on serovar rather than host, however, isolates of the same serovar/ST type could be separated into different branches/clades using more discriminatory methods (i.e., SNP typing).

Virulence genes and SPI

- Isolates within a serovar from different sources had similar virulence gene / SPI profiles.
- Within a serovar, several virulence genes / SPI were shown to be more prevalent in humans, while others were more prevalent in animals.
- Likewise, several genes / SPI were shown to be highly associated with some serovars but not others.

Antimicrobial resistance genes

- AMR genes were detected in very low percentages of the *Salmonella* isolates, with most AMR genes absent from most animal derived strains.

5.2 Benefits to industry

The project facilitated the development of a genomics capability that will serve industry to better understand and manage risks associated with *Salmonella*, while supporting industry to meet future needs associated with molecular risk-based schemes. The software analysis workflow developed for the rapid characterisation of *Salmonella* can be adapted or modified to evaluate future international developments around molecular based risk characterisation methods. In addition, the large database of *Salmonella* sequences generated in this project will serve as a valuable resource that can be interrogated to provide updated information on risk, as molecular risk approaches evolve. Likewise, data can be used to inform risk management practices that in turn minimise the reputational, trade and public health risks associated with contaminated beef entering domestic and export markets.

6. Future research and recommendations

The project provides an initial understanding of the risk of Australian *Salmonella* in the red meat industry based around a subset of historical isolates. Future activities should be designed to build on this by providing additional evidence for managing risks and it is recommended that:

- Further attempts should be made to establish collaborations with US-based researchers to access propriety information on HPS and DENT. Such information would provide foresight

into how Australian *Salmonella* isolated from red meat supply chains perform against potential alternative performance standards.

- Collaborations should be pursued with local public health labs to gain access to human isolate metadata for improved risk characterisation.
- Comparisons should be conducted to determine if Australian *Salmonella* sequences align with international *Salmonella* sequences, particularly those in destination markets for Australian beef.
- Rapid systems should be trialled for the semiquantitative or quantitative detection of high-risk *Salmonella*.
- Additional surveys of *Salmonella* could be undertaken across a broader range of sample sites (e.g., hides and lymph nodes) to identify potential carcase contamination pathways for priority serovar groups.
- Assessing the performance of bioinformatics-based typing methods against traditional 'gold standard' characterisation tools or commercial test methods for identifying HPS and DENT (when / if they become available). This could also include aligning bioinformatics approaches with national and international approaches to improve confidence in results.

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8. Appendix

8.1 MLST Sequence Types (ST)

Predicted Serovar	Host	Count	ST (MLST Achtman scheme)
Aberdeen	animal	5	426 (100%)
	human	1	426 (100%)
Agona	animal	20	13 (100%)
	human	21	13 (100%)
Anatum	animal	60	64 (100%)
	human	70	64 (100%)
Bovismorbificans	animal	56	377 (94.6%), 1499 (3.6%)
	human	66	377 (89.4%), 1499 (9.1%), 142 (1.52%)
Chester	animal	14	343 (64.3%), ND (35.7%)
	human	15	343 (73.3%), 3596 (13.3%), 2063 (6.7%), ND (6.7%)
Derby	animal	1	71 (100%)
	human	3	40 (100%)
Dublin	animal	3	10 (100%)
	human	17	10 (88.2%), 4293 (5.9%), ND (5.9%)
Enteritidis	human	16	11 (100%)
Gallinarum or Enteritidis	animal	1	180 (100%)
Heidelberg	animal	8	15 (100%)
	human	5	15 (100%)
Hvittingfoss	animal	2	434 (50%), 446 (50%)
	human	3	4293 (66.7%), 446 (33.3%)
Infantis	animal	26	32 (92.3%), ND (7.7%)
	human	37	32 (97.3%), ND (2.7%)
Kottbus	animal	3	1792 (100%)
Litchfield	animal	1	4491 (100%)
	human	1	214 (100%)
Mbandaka	animal	17	413 (100%)
	human	4	413 (75%), 1602 (25%)
Montevideo	animal	16	138 (100%)
	human	2	138 (50%) 316 (50%)
Muenchen	animal	25	82 (96%), 3211 (11%)
	human	5	82 (100%)
Newport	animal	3	31 (66.7%), ND (33.3%)
	human	19	31 (79%), 4166 (5.3%), 45 (5.3%), 46 (5.3%), 166 (5.3%)
Poona	animal	3	1069 (100%)
	human	4	1069 (100%)
Reading	animal	21	93 (100%)
	human	3	93 (100%)
Saintpaul	animal	58	50 (89.7%), ND (10.3%)
	human	53	50 (92.2%), 27 (3.8%)

Singapore	animal	1	462 (100%)
	human	1	462 (100%)
Thompson	human	8	26 (75%), 2125 (12.5%), ND (12.5%)
Typhimurium	animal	164	19 (98.8%), ND (1.2%)
	human	187	19 (89.3%), 2089 (7.5%), 213 (0.5%), 34 (0.5%), 36 (1.6%), 5060 (0.5%)
Virchow	animal	15	16 (100%)
	human	16	16 (93.8%), 197 (6.3%)
Wangata	animal	1	523 (100%)