

A multi-jurisdictional outbreak of *Salmonella* Typhimurium infections linked to backyard poultry—Australia, 2020

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Abstract

Zoonotic salmonellosis can occur either through direct contact with an infected animal or through indirect contact, such as exposure to an infected animal's contaminated environment. Between May and August 2020, a multi-jurisdictional outbreak of *Salmonella* Typhimurium (STm) infection due to zoonotic transmission was investigated in Australia. In total, 38 outbreak cases of STm with a median age of 5 years were reported. Epidemiological investigation showed contact with live poultry to be a common risk factor with most cases recently purchasing one-week old chicks from produce/pet stores. Traceback investigation of cases identified 25 product/pet stores of which 18 were linked to a single poultry breeder farm. On farm environmental sampling identified the same STm genotype as identified in cases. Whole genome sequencing of both environmental and human outbreak isolates found them to be highly related by phylogenetic analysis. This investigation describes the first documented widespread zoonotic salmonellosis outbreak in Australia attributed to backyard poultry exposure and identified potential risk factors and prevention and control measures for future outbreaks. Prevention of future outbreaks will require an integrated One Health approach involving the poultry industry, produce/pet store owners, animal healthcare providers, public health and veterinary health agencies and the public.

KEYWORDS

backyard poultry, outbreak, *Salmonella* Typhimurium, salmonellosis, whole genome sequencing, zoonosis

1 | INTRODUCTION

Salmonella, and its various serotypes, represent the greatest foodborne burden worldwide (Kirk et al., 2015). Although the majority of *Salmonella* infections are foodborne, a report from the United States estimated that 11% of non-typhoidal salmonellosis can be

attributed to infected animal contact (Hale et al., 2012). Outbreak investigations of zoonotic salmonellosis have demonstrated that infected animals, such as live poultry in the home, present a high risk for transmission. Infection can occur either through direct contact or through indirect contact, such as exposure to the infected animal's contaminated environment (Basler et al., 2016).

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Salmonella Typhimurium (STm) is the most frequently notified human *Salmonella* serotype in Australia. There have been, on average, 5900 cases notified each year in Australia between 2010 and 2017 (Australian Government Department of Health, 2021). To identify potential clusters of STm infection, Australian jurisdictions subtype isolates using either Multiple Locus Variable-Number Tandem Repeat Analysis (MLVA) or whole genome sequencing (WGS). To enable the detection of potential outbreaks and link cases in multi-jurisdictional investigations, isolates identified as clustering by MLVA are subsequently analysed by WGS.

OzFoodNet is an Australian network that conducts surveillance on foodborne diseases. In May 2020, Queensland's OzFoodNet site was notified by the Queensland Health (QH) Public Health Microbiology laboratory of six cases of STm MLVA 03-11-10-08-523 with specimen collection dates clustered over a one-week period. There had only been three cases of STm MLVA 03-11-10-08-523 reported in this jurisdiction in the previous 5 years. A further three cases were reported the following week. This report describes the investigation of a multi-jurisdictional STm outbreak linked to live poultry in Australia.

2 | MATERIALS AND METHODS

2.1 | Epidemiological, environmental and initial laboratory investigation

A case was defined as any person notified between 1 May 2020 and 31 August 2020 with a laboratory-confirmed diagnosis of STm MLVA 03-11-10-08-523 or closely related MLVA variant genomically highly related on phylogenetic analysis (<4 single nucleotide polymorphisms [SNP] difference) to case isolates in the same core genome multilocus sequence typing (cgMLST) cluster complex. Each jurisdiction investigated their respective cases using telephone administered hypothesis-generating questionnaires to collect demographic, clinical and risk factor information. Produce stores identified from case interviews were investigated using a telephone administered standard questionnaire. Details of produce stores' poultry suppliers were obtained either by produce store investigations or by review of the produce stores that were supplied by the poultry breeder farm as detailed on the poultry breeder farm website. Monitoring of *Salmonella* notifications with the same MLVA type as the outbreak strains continued after the implementation of prevention and control measures to determine control of the outbreak.

The environmental investigation focussed on the source (farm) as limited resources prevented further environmental sampling at the homes of cases. However, QH was able to intensively sample the household of one case. On 8 June 2020, QH collected environmental samples from the chicken coop at the property of a case. Additionally, on 16 June 2020 QH and Biosecurity Queensland (BQ, the government agency managing pest and disease threats to agriculture, the environment, social amenity and human health) conducted a joint initial onsite farm assessment and sampling at the poultry breeder farm. BQ conducted further follow up assessments

Impacts

1. First documented widespread zoonotic salmonellosis outbreak in Australia attributed to backyard poultry.
2. Linking epidemiology and whole genome sequencing to investigate *Salmonella* outbreaks.
3. An integrated One Health approach is needed to prevent salmonellosis associated with backyard poultry.

on 9, 10, 13 and 16 July 2020 with additional environmental samples collected on 10 and 13 July 2020. On-site farm assessment consisted of verbal questioning of staff, review of standard operating procedures and the Hazard Analysis and Critical Control Point (HACCP) plan and a guided farm tour detailing the flow of operations. On-site sample collection was undertaken across the farm and included five poultry sheds and the hatchery.

All environmental samples collected during the investigation were submitted to the QH Public Health Microbiology laboratory for PCR, culture and further microbiological characterization as required (serotyping, MLVA and WGS).

Although usually not considered suitable for PCR due to the presence of inhibitory compounds that interfere with the PCR reaction, faeces, drag swabs, disposable booties, environmental swabs and feed collected from the breeder farm were subjected to both real-time PCR and culture to provide rapid turnaround of results and implementation of corrective action where necessary.

Feed and faeces were added to a volume of buffered peptone water (BPW), a non-selective enrichment broth to achieve a 1 in 10 proportion. Environmental swabs were inserted into a tube containing 10 mL of BPW. Disposable booties were placed into a jar and submerged in sufficient BPW to cover the boots. All samples were incubated overnight at 37°C (Standards Australia, 2002).

Subculture into selective broths consisted of subculture of 1 ml of BPW into 10 ml mannitol selenite cystine broth (MSCB) incubated at 37°C for 24 hr and 100 µl BPW into each of two 10 ml tubes of Rappaport-Vasiliadis soya (sRV) peptone broth with one tube incubated at 42°C and the other at 44°C, both for 24 hr. The inclusion of sRV tube at 44°C can provide improved recovery of salmonellae in heavily contaminated samples (in-house addition to the AS standard). All tubes were subcultured onto xylose lysine deoxycholate (XLD) agar (37°C/24 hr) and bismuth sulphite (BS) agar (37°C/48 hr).

Agar plates were examined for typical colony morphology and confirmed using the Bruker MALDI-TOF (matrix-assisted laser desorption/ionization time of flight) Biotyper. Confirmed colonies were subcultured onto nutrient agar slopes with 1 ml of sterile water added to stimulate flagellum growth. After incubation at 37°C for 24 hr, the inoculated slopes were submitted for serotyping. Isolates confirmed as STm were submitted for MLVA.

Salmonella isolates from human specimens were isolated in diagnostic pathology laboratories and referred to jurisdictional public health reference laboratories for serotyping and further characterization.

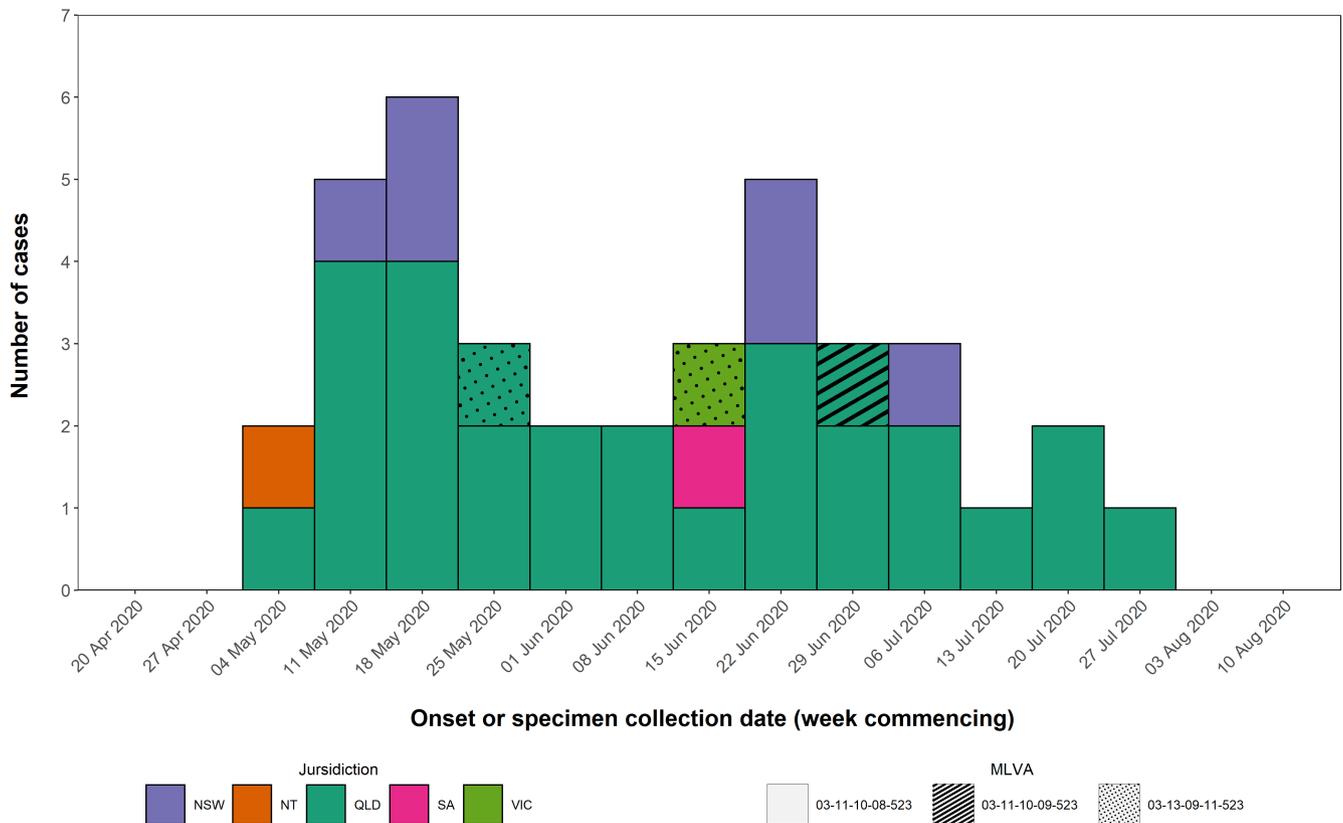


FIGURE 1 Epidemic curve of STm cases by week of onset or specimen collection date by jurisdiction and MLVA type, 20 April to August 10, 2020 [$n = 38$]. Note: Specimen collection date was used as a proxy for three QLD cases with unknown onset dates of illness (29 May, 1 June, June 22, 2020)

For MLVA typing, the procedure of Lindstedt et al. (2004) was followed with minor modifications: the concentration of primers for STTR6 and STTR9 was halved, the size marker was labelled with ROX, and fragment sizing was performed on an ABI 3130 sequencer (Lindstedt et al., 2004). MLVA profiles were expressed as a number string of allele repeat numbers (as generated by the sequencer) for each allele in the order STTR9-STTR5-STTR6-STTR10pl-STTR3.

Salmonella isolates of human and non-human source were serotyped using antisera and the antigenic formulae from the Kauffman-White scheme (Popoff et al., 2000).

2.2 | Molecular laboratory investigation

Sequences for both human and non-human STm isolates were generated using the Illumina NextSeq500 genomic sequencing platform at the QH Public Health Microbiology laboratory. DNA was extracted from cultures using the QiaSymphony DSP DNA Mini kit (Qiagen) according to the manufacturer's protocol. DNA was prepared for sequencing using the Nextera XT kit (Illumina) and sequenced on the NextSeq500 using the NextSeq 500 Mid Output v2 kit (300 cycles) (Illumina) according to the manufacturer's instructions. Genetic relatedness among STm isolates was investigated by cgMLST using Ridom SeqSphere+ (v6.0.0) based on the Enterbase *S. enterica* scheme, and by SNP analysis using the snippy package (V4.3.6),

with *Salmonella* Typhimurium LT2 as a reference (Genbank accession number NZ_CP014051). A maximum likelihood tree was constructed from the core SNPs using the RAxML plugin in Geneious R11.

To provide context, 11 unrelated historical STm MLVA 03-11-10-08-523 human isolates reported from Queensland between December 2005 and February 2020 were also included in the analysis.

2.3 | Ethics

This investigation was conducted as part of the public health response to an acute outbreak in accordance with the respective jurisdictional public health legislation, and as such, ethics approval for this work was not required.

3 | RESULTS

3.1 | Epidemiological, environmental and initial laboratory investigation

In total, there were 38 laboratory-confirmed cases associated with this outbreak. Onset dates of illness among confirmed cases ranged from 4 May 2020 to 29 July 2020. The 38 cases were reported from Queensland (QLD) ($n = 29$), New South Wales (NSW)

($n = 6$), Northern Territory (NT) ($n = 1$), South Australia (SA) ($n = 1$) and Victoria (VIC) ($n = 1$) (Figure 1). Among the cases, 35 were STm MLVA 03-11-10-08-523, two were 03-13-09-11-523, and one was 03-11-10-09-523 (Figure 1).

The median age of cases was 5 years (range: <1–38 years [$n = 38$]); 31 (81%) were aged 14 years or younger. Twenty-four cases (63%) were male. There were 36 (95%) case interviews completed and 9 (25%) cases were hospitalized; the median time spent in the hospital was 4 nights (range: 1–5 nights). Diarrhoea was present in 100% (33/33) of interviewed cases where information was available, abdominal cramps in 96% (22/23), fever in 81% (22/27), bloody diarrhoea in 67% (18/27) and vomiting in 42% (11/26). Median duration of symptoms reported among cases was 8 days (range: 3–15 days [$n = 19$]).

All 38 cases reported direct contact with live poultry with 37 cases reporting exposure to backyard poultry in the week prior to onset of illness. Cases exposed to backyard poultry also reported handling the poultry and described behaviours such as touching, hugging, kissing and housing poultry inside their homes. Of those exposed to backyard poultry, 33 (89%) had recently purchased one-week-old chicks and 31 (84%) were able to provide trace-back information on place of purchase of chicks. The investigation identified 25 separate produce/pet stores where poultry were purchased by cases. Of these, 18 (72%) stores had been supplied poultry from the same breeder farm. Information on the poultry supplier for the other 7 produce/pet stores was not obtained. One case reported no exposure to backyard poultry; however, the case worked as a farmhand at a broiler farm located near the linked poultry breeder farm.

On-site assessment of the poultry breeder farm identified that the farm had their own genetic breeding stock which reduced the need for externally sourced poultry. The assessment also found extensive contamination across the hatchery incubators, such as soiled eggs and dirty circulating fans which may have led to ineffective fumigation. Poor vermin management was suggested by structural issues in shed screening, and live and dead vermin inside and outside of the shed. In addition, recent changes to staffing and standard operating procedures were noted. This staff turnover led to reduced application of the new standard operating procedures and the HACCP plan. As a result, maintenance and cleanliness of the farm was not consistently achieved.

A total of 77 samples were collected from the poultry breeder farm by QH and BQ on 16 June, 10 July and 13 July 2020. Samples from the poultry farm included surface swabs from the hatching room, feeders, water lines and elevated slats/roosts, in addition to boot covers, drag swabs and feed. *Salmonella* with the same MLVA types as the outbreak cases (STm MLVA 03-11-10-08-523 and STm MLVA 03-13-09-11-523) were detected in 13 different environmental farm samples taken from the hatchery and multiple poultry sheds (Table 1). *S. Agona*, *S. Cerro*, and *S. Hvitvingfoss* were also detected in four different environmental farm samples. These additional serotypes were not associated with illness in humans and therefore did not undergo further characterization.

TABLE 1 Positive *Salmonella* samples by date, location, sample description, serotype and MLVA

8 June 2020: Home of case	
Chicken breeding box: Chicken faeces	STm MLVA 03-11-10-08-523
16 June 2020: Poultry farm	
Hatchery shed setter rooms: Boot swab	<i>S. Hvitvingfoss</i>
Hatchery shed setter rooms: Drain swab	STm MLVA 03-11-10-08-523
Shed 10: Boot swab	STm MLVA 03-26-14-11-523
	<i>S. Agona</i>
Shed 10: Drag swab	STm MLVA 03-26-14-11-523
Shed 10: Waterline swab	STm MLVA 03-11-10-08-523
Shed 11: Boot swab	STm MLVA 03-11-10-08-523
	<i>S. Agona</i>
	<i>S. Cerro</i>
Shed 11: Drag swab	STm MLVA 03-13-09-11-523
Shed 12: Boot swab	STm MLVA 03-11-10-08-523
10 July 2020: Poultry farm	
Rearing shed: Drag swab	STm MLVA 03-11-10-08-523
13 July 2020: Poultry farm	
Hatchery: Incubator (wall) swab	STm MLVA 03-13-09-11-523
Hatchery: Incubator (wall) swab	STm MLVA 03-11-10-08-523
Hatchery: Incubator (floor) swab	STm MLVA 03-11-10-08-523
Hatchery: Incubator (floor) swab	STm MLVA 03-11-10-08-523
Hatchery: Incubator (chick trays) swab	STm MLVA 03-11-10-08-523
Hatchery: Incubator feathers	STm MLVA 03-11-10-08-523
Day-old chicks: One-day-old chicks	STm MLVA 03-11-10-08-523

Eight samples were collected from the home of a case linked to the outbreak by QH on 8 June 2020. Samples from the home included surface swabs of the chicken coop and inside a breeding box. One of the eight samples tested positive for *Salmonella*. This sample had the same MLVA type as the most common outbreak strain (STm MLVA 03-11-10-08-523).

Increased prevention and control measures were put in place at the poultry farm in response to the outbreak to reduce the likelihood of future *Salmonella* outbreaks. These included the administration of a STm vaccine to the breeder flock and increased biosecurity measures such as water chlorination, installation of hand/foot washing facilities, fumigation of sheds between batches, increased frequency of routine microbiological testing and increased vermin bait stations. Health-related fact sheets developed through the collaboration of QH and BQ on poultry and *Salmonella* and ways to minimize infection in people were made available on health and agriculture department websites and distributed to produce/pet stores. A media statement was also released by QH on 2 July which provided details of the outbreak, information on the risks of zoonotic salmonellosis and public

health advice, including personal hygiene behaviours, for owners of backyard poultry.

Notifications rapidly declined to below-expected numbers after 13 July 2020, and subsequent investigations of these cases did not identify a link to backyard poultry.

3.2 | Molecular laboratory investigation

A total of 19 STm MLVA 03-11-10-08-523 isolates (13 human; 6 environmental) associated with this outbreak were submitted for WGS. The 19 STm MLVA 03-11-10-08-523 isolates associated with this outbreak were genotyped as cgMLST complex type 5922, and all clustered together within 0–3 SNPs (Figure 2). The 11 geographically and temporally unassociated STm MLVA 03-11-10-08-523 isolates included in the analysis for context were not highly related to this cluster, with >19 SNPs present between these isolates and those in the cluster.

Two STm MLVA 03-13-09-11-523 isolates (1 human; 1 environmental) were detected in this outbreak and were submitted for WGS. Both isolates were genotyped as cgMLST complex type 3454, with only one SNP difference detected. These two isolates also clustered with isolates from a previously reported outbreak of STm 03-13-09-11-523 in 2019 (cgMLST 3454, 0–7 SNP difference) that was linked to a chick hatching programme with the fertile eggs used in this programme being supplied by the same poultry breeder farm (OzFoodNet Queensland, personal communication, August 2020).

4 | DISCUSSION

Our investigation provided very strong evidence to implicate the poultry breeder farm as the source of infection for this outbreak. This included microbiological evidence showing the detection of the same STm genotype in swabs taken at the poultry breeder farm as that found in human cases and the traceback investigation which

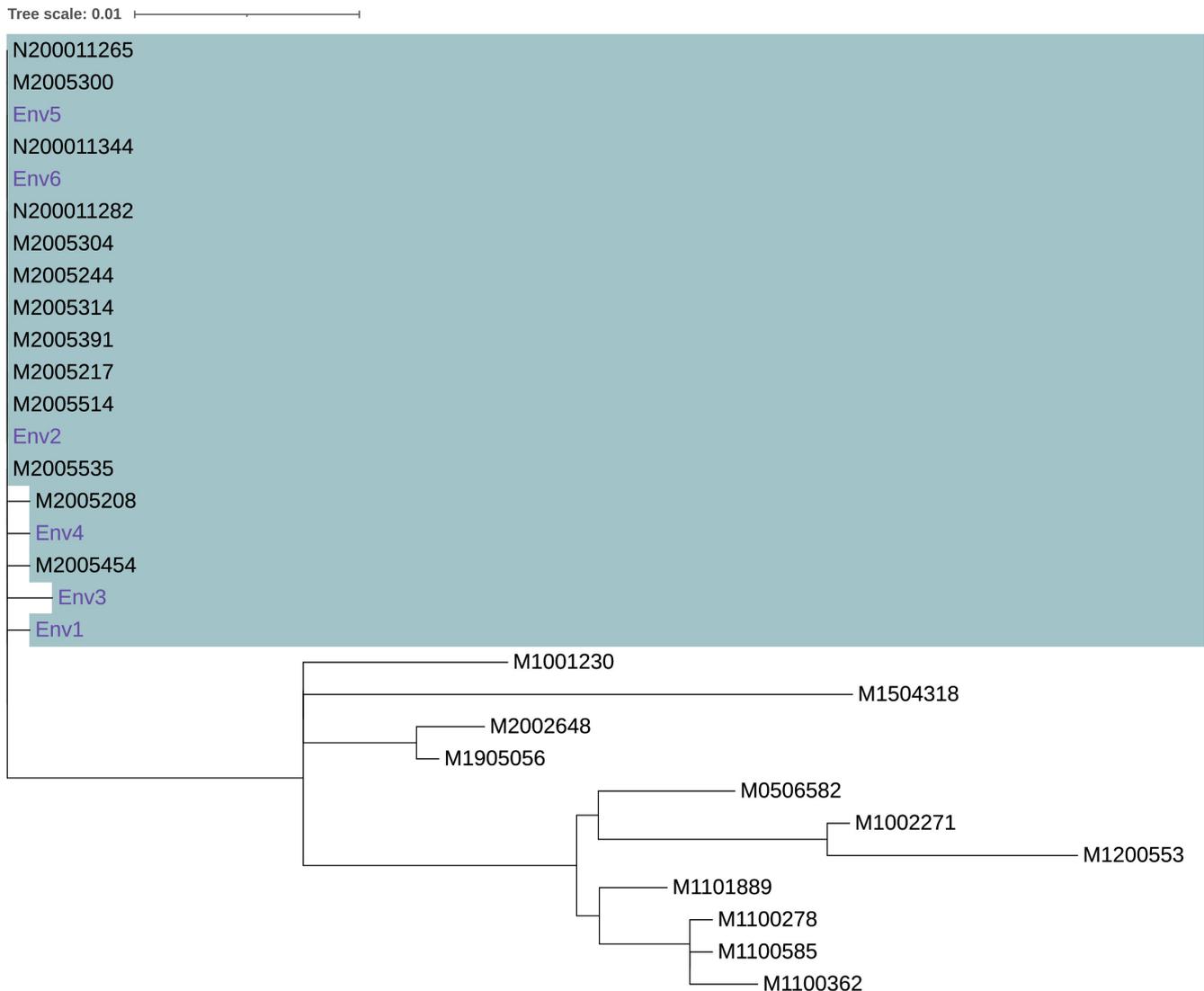


FIGURE 2 Maximum likelihood tree built using SNP differences. The cluster identified is highlighted in blue. Labels for environmental isolates are shown in purple and labels for clinical isolates are shown in black. The length of branches represents distance

linked cases to the poultry breeder farm through the produce/pet stores that supplied the one-week-old chicks. Transmission was most likely through the direct contact and handling of one-week-old chicks sold through retail produce/feed and pet stores. In addition, poor human-animal handling factors in the home and limited on-farm biosecurity measures, which may have been partly driven by increased pressure on production due to increased demand, is the most likely explanation for the widespread nature of this outbreak.

This is the first documented widespread *Salmonella* outbreak linked to backyard poultry identified in Australia, but similar outbreaks have been reported internationally (Nicols et al., 2021). In the United States, cases of salmonellosis in owners of backyard poultry have been linked to both agricultural feed stores and mail-order hatcheries (Anderson et al., 2016; Robertson et al., 2019). A review of live poultry-associated salmonellosis outbreaks in the United States showed that the median age of cases was 9 years and identified high-risk practices included keeping poultry inside households and touching, snuggling and kissing birds (Basler et al., 2016). Previous reports have highlighted the need for health-related information to be provided at the point of purchase (Anderson et al., 2016).

Management practices at poultry production systems may influence the transmission and persistence of salmonellae and potentially contaminate end of line products (Liljebjelke et al., 2005). Results of the on-site assessment suggested poor biosecurity practices which likely contributed to the persistence of STm at the poultry farm and its spread beyond the farm. To reduce the incidence of *Salmonella* colonization at the farm-level, several preventative and monitoring strategies are required. Vaccination is only partly effective for controlling *Salmonella* and must be combined with preventative hygienic behaviours such as water treatment and *Salmonella* free breeding flocks, housing, feed and litter (Vandeplas et al., 2010). Additionally, further measures may be required, such as dietary modification, to effectively control this organism on farm (Doyle & Erickson, 2006). In response to the outbreak, the farm increased biosecurity measures to help control and prevent future salmonellosis outbreaks. Increased routine monitoring will aid in its early detection and may suggest the modification or addition of on-farm *Salmonella* preventative and control measures.

The popularity of backyard poultry ownership has increased in recent years (Robertson et al., 2019). Of note, this outbreak occurred shortly after SARS-CoV-2 was first identified in Australia in 2020; a time when there were reports of a sudden demand for backyard chickens (Gorman, 2020; Groves, 2020). A similar purchasing trend most likely driven by COVID-19 was observed in the United States along with an increase in *Salmonella* outbreaks (Nicols et al., 2021). Australian reports have shown that biosecurity practices of small poultry keepers are poor compared with commercial enterprises (Hernández-Jover et al., 2014) and that backyard poultry owners also have limited contact with veterinarians, which may place them at higher risk of zoonotic disease transmission. The combination of increased zoonotic disease risk to backyard poultry owners and increase in backyard poultry ownership represents a future risk of widespread zoonotic salmonellosis outbreaks.

Cross-discipline collaboration and sharing of technical advice between public health and veterinary health agencies is required to provide accurate information on zoonotic salmonellosis and prevent, detect and control future outbreaks in owners of backyard poultry. From a disease control perspective, it is important to make information available and easily accessible using multiple communication channels. Several guidelines have been released in Australia by state departments of agriculture and industry bodies detailing legislative and biosecurity requirements which target owners of backyard poultry (Australian Eggs, 2021; Government of Western Australia Department of Primary Industries and Regional Development, 2018). These guidelines outline ways to manage poultry in the backyard, who to contact when poultry show signs of disease, and how to reduce risk of disease spread. In response to this outbreak, fact sheets were developed and made available for both the public and for staff of produce/pet stores to assist in the prevention of transmission of salmonellae in humans and published on health and agriculture department websites. Additionally, and specific to this outbreak, a media statement was released to bring awareness of the outbreak to the public and provide advice to owners of backyard poultry (Queensland Government Queensland Health, 2020). Although it is not possible to know whether owners of backyard poultry used this information during the outbreak, the outbreak investigation team used multiple methods of communication to bring awareness of the risk of zoonotic salmonellosis to help in controlling the spread of this and future outbreaks.

Collaboration between public health and veterinary health agencies contributed to the control of this outbreak. QH and BQ investigated cases, produce/pets stores and the linked poultry farm to gather epidemiological and laboratory evidence in addition to implementing prevention and control measures to manage the outbreak. The successful control of this outbreak was achieved through the sharing of data. An integrated One Health approach is required to prevent and control zoonotic disease outbreaks (Wendt et al., 2015). Improvements in public health surveillance and outbreak detection and control can be achieved by linking epidemiologic and WGS data (Vaughn et al., 2020). In addition, having clearly defined roles and responsibilities at the organizational level aids with ongoing engagement, operations and sharing of data between agencies (Errecaborde et al., 2019). Moving forward, prompt linkage of epidemiological and molecular data to detect clusters, assessment of spread and confirmation of the source of infection are recommended for zoonotic salmonellosis outbreaks.

Information to identify the poultry supplier for seven of the produce/pet stores was not obtained. In addition, only two of three outbreak strains were isolated from environmental samples collected at the identified poultry supplier. This may have led to contributors of disease spread, such as more than one poultry supplier, not being identified. However, the outbreak was controlled with remedial action at the single poultry provider identified through traceback and molecular investigation.

This investigation describes the first documented widespread zoonotic salmonellosis outbreak in Australia attributed to live poultry

exposure and has identified potential risk factors and prevention and control measures for future outbreaks. Backyard poultry ownership increases the risk of human salmonellosis but is largely preventable. The prevention of future outbreaks will require an integrated One Health approach which will need to include the poultry industry, the public, backyard poultry owners and human and animal health officials.

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CONFLICT OF INTEREST

No conflict of interest to declare.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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