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The use of whole genome sequence typing (WGST) for traceability in an unusual outbreak of *Salmonella enterica* subspecies *enterica* serotype Enteritidis (*S.* Enteritidis) in Victoria, Australia.

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Introduction
Salmonella enterica subspecies *enterica* serotype Enteritidis (S. Enteritidis) is one of the most common causes of foodborne gastroenteritis in humans worldwide. Traditionally cases of human illness caused by S. Enteritidis in Australia have been associated with returned travellers and the consumption of contaminated animal-based food products overseas. Until 2018 S. Enteritidis was considered an exotic disease in Australian poultry. In May 2020, a small cluster of locally acquired human cases of S. Enteritidis were identified in Victoria, Australia, with a likely link to backyard layer hens, either through direct contact or consumption of eggs. Commercial egg farms often sell birds at the end of the production cycle, known as spent hens to backyard holders.
It is not unusual to find Salmonella in poultry farms, the significance of these findings depends on accurate identification and strain typing, which are critical to the success of source tracing and tracking following a public health incident. Successful identification and tracing allow for prompt employment of quarantine and preventative measures to prevent disease spread. Until recently, screening environmental samples for Salmonella from commercial/backyard poultry/egg farms used traditional culturing technologies. Here we describe how epidemiological data in combination with WGST was used to establish links between human cases and the identified likely source.
Methods
Agriculture Victoria Biosecurity Officers conducted interviews to follow potential traces from each public health case. A resulting 54 properties were identified for sampling and swabs were submitted for laboratory testing using culturing technologies. Any pure Salmonella colonies were further investigated using whole genome sequence typing (WGST). Genomic DNA was extracted from pure colonies and short read sequencing performed on an Illumina Miseq®/Novaseq® instrument. The sequencing data was used for Multilocus sequence typing (MLST) and whole genome phylogenetic analysis were used to determine likely linkages between isolates taken from multiple sources and establish connections.
Results
54 properties were examined for the presence of S. Enteritidis throughout this study. Each property comprised multiple sampling regimes over twelve months. Of the 54 properties eight had S. Enteritidis detected with a total of 15 S. Enteritidis isolates sequenced. Of the 46 remaining properties, 18 different Salmonella spp serotypes were isolated. The MLST analysis revealed all positive isolates as ST 11, the same strain found in the locally acquired human cases and highly related to the 2019-001 MJOI strain of S. Enteritidis that contaminated multiple New South Wales egg farms in 2018, 2019 and 2020 and a single Victorian egg farm in March 2019.
Conclusions
In this report, we describe how a cluster of human Salmonellosis cases uncovered an unusual outbreak of S. Enteritidis in backyard chickens in Victoria and the use of WGS to reveal epidemiological linkages. This paper highlights the complexities that exist with a disease that does not always present clinically in hens, can sit unrecognised in a backyard flock until it presents in human disease and how the unregulated onward sale of spent hens can complicate the containment of a zoonotic and notifiable disease in poultry.