On-farm Control of *Campylobacter* in Broiler Poultry

Lay summary prepared by Joanne M. Kingsbury, 26 August 2021

Core project team: Patrick Biggs, Anne C. Midwinter, Nigel French, Jackie Benschop, Rebecca Lucas-Roxburgh, Joanne M. Kingsbury, Nicola King, Maree Callander, Kerry Mulqueen, Michael Brooks, Roy Biggs

Peer review: Peter Cressey and Catherine McLeod

Campylobacteriosis is the most frequently notified enteric disease in New Zealand. Voluntary biosecurity interventions by the poultry industry, including changes to slaughter and processing practices, together with the implementation of the Ministry for Primary Industries' *Campylobacter* Risk Management Strategy, led to a ~50% reduction in incidence during 2006-2008. Although the proportion of poultry-associated cases has decreased, poultry remains an important source of human infection. To reduce cross-contamination during processing, reliance on processing interventions, and contamination of product, it would be preferable to reduce *Campylobacter* colonisation of poultry at the farm level. This study aimed to provide a better understanding of on-farm sources for *Campylobacter* contamination of New Zealand broiler flocks.

The study was structured into two workstreams. **Phase 1** included a **literature review**, the development of a ***Campylobacter*-specific metabarcoding method** and a **limit of detection (LOD) laboratory study**. These three elements all informed on the design of the **Phase 2 longitudinal broiler farm microbiological survey**.

The **literature review** assessed recent literature regarding important reservoirs, pathways and risk factors for *Campylobacter* contamination of broiler farms and flocks. Some key findings from the review were:

- There are multiple important reservoirs, pathways, and risk factors, which may differ from farm-to-farm. This makes controlling *Campylobacter* in flocks challenging.
- Worker movement into the sheds is considered the most important transmission route for *Campylobacter* ingress into sheds.
- There is increased risk of *Campylobacter* contamination when there has been a *Campylobacter*-positive flock in a broiler house on the farm, and when there are neighbouring broiler farms.
- Other nearby livestock, wildlife, pets and insects can be vectors but the direction of transmission is not always clear between these animals and the poultry.
- There is limited evidence for transmission from the breeder flock, feed, air, litter or drinking-water (although contaminated standing water around the farm or biofilms in the shed drinking-water system might be a problem).

A ***Campylobacter*-specific, metabarcoding method** was developed as a complementary approach to Whole Genome Sequencing (WGS) for the analysis of on-farm study samples. Metabarcoding is a culture-independent method that involves amplification of a short genetic region (the amplicon) from the organism of interest (i.e., *Campylobacter*) within a sample. This is followed by DNA sequencing of the generated amplicon (or amplicons) and comparison of sequence reads to a reference database to provide a more comprehensive picture of the *Campylobacter* types and their prevalence in samples. Metabarcoding allows for the detection

of viable but not culturable cells, sequence types (STs)[1] that are present in low numbers in mixed populations, and slower-growing STs that might be outcompeted during culture; thereby, increasing the potential for making linkages between *Campylobacter* in sources and birds. Development of the method involved selecting candidate amplicon regions from a dataset of 1,343 genes present in all *C. jejuni* and *C. coli*, focusing on those which have a high level of sequence diversity between different *Campylobacter* species and genotypes. The three most informative candidate amplicons were selected following performance testing, and sample processing methods were optimised.

An **LOD laboratory study** tested and refined testing methodology for some of the proposed sample types and processing methods for inclusion in the Phase 2 survey. Most of the sample types collected in the study are not usually tested by the testing laboratory involved in the study, and testing methods for some of these types are not well defined.

The **Phase 2 longitudinal broiler farm microbiological survey** followed a single broiler flock from the parent flock, hatching, through the entire life of the flock, to processing. The survey was timed to coincide with peak *Campylobacter* seasonal prevalence and took place during October to December 2019. Multiple samples were taken at frequent sampling intervals of potential *Campylobacter* reservoirs and sources (e.g. environmental sources such as wild birds, the farm and shed environment, the previous flock inhabiting the shed, another flock of the same age, and the breeder flock), potential transmission routes for *Campylobacter* ingress into the broiler shed (e.g. farm workers, catching crews and equipment, rodents and insects), and the target flock, to monitor *Campylobacter* colonisation of the flock (cloacal swabs of live birds, caecal contents and carcass rinsates of birds during processing).

Of the 738 samples tested, 200 (27%) tested positive for *Campylobacter* by cultural isolation. The species was determined for up to four isolates per positive sample; the species breakdown included 316 *C. jejuni*, 39 *C. coli* and 8 *C. lari* isolates. The previous flock in the shed tested positive for *C. jejuni*, but *Campylobacter* was not isolated from the shed following cleanout. All samples from the study flock, as well as environmental samples from inside the broiler shed, remained *Campylobacter*-negative until the after the first cut; at which time, a high proportion of catcher and catching equipment samples tested positive (12/27, 44% at first cut; and 59/130, 45% of total catcher samples). After that time (by 35 days), a high proportion of birds and shed samples (including boot, and surface and drinker swabs, insect samples and drinkers) tested positive for *Campylobacter*.

While *C. jejuni, C. coli* and *C. lari* were all identified from environmental samples, *C. jejuni* was the only species also isolated from chickens. A total of 199 *C. jejuni* isolates were sequenced and data was analysed to link *Campylobacter* isolates from sources, reservoirs, and transmission routes with chicken isolates. Significant findings include:

- The isolates comprised seven STs; ST50, ST45, ST3105, ST3663C, ST25, ST53 and ST6964. ST6964 was the most abundant (105 isolates). This fluoroquinolone and tetracycline-resistant ST is one of the most frequently isolated from New Zealand poultry flocks in recent years, as well as from human cases.
- Most isolates from the study flock were ST6964 (44 isolates) or ST50 (27 isolates). These closely matched 11 ST6964 isolates and one ST50 isolate from the previous flock residing

[1] Sequence typing is a technique for strain characterisation which uses the nucleotide sequences from several conserved housekeeping genes to derive a combination of alleles; strains comprising a single ST share identical allelic profiles.

in the shed, and isolates from an age-matched control flock on the same farm. Results support a role for carry over from the previous flock or another on-farm reservoir in contaminating the current flocks.

- There were six different STs among catching crew and equipment isolates. The most prevalent were ST6964 (19 isolates) and ST50 (21 isolates), many of which also closely matched, or were indistinguishable from, chicken isolates. The close genetic match, high *Campylobacter* prevalence in catcher samples, and the timing of flock infection occurring closely following catcher presence in the shed, also support that catchers and equipment might cross-contaminate the shed and flock from prior flocks that they visited. Results are consistent with the literature review findings that worker movement into the sheds is an important transmission route for *Campylobacter* ingress into sheds.
- There was no evidence for wildlife (e.g., rabbits, wild birds, or insects), feed, drinking-water or the shed litter as sources of the *Campylobacter* genotypes colonising the broiler flock.

A selection of 384 pre- or post-enrichment broths from 367 samples (prioritised in part by collection prior to the flock becoming colonised by *Campylobacter*) were also tested using the *Campylobacter*-specific metabarcoding method developed in Phase 1. Metabarcoding analyses were in accordance with WGS-based results but also provided new insights; key findings from the metabarcoding component include:

- In samples from which *Campylobacter* was isolated and metabarcoding performed, metabarcoding detected most of the *Campylobacter* STs that isolation did, plus additional STs which were not isolated. For example, *C. jejuni* ST257 was commonly detected by metabarcoding, including at a high abundance from the previous and current flock, but was never isolated from samples. These types might be outcompeted during culture by more commonly isolated STs, or are present at lower levels in the environment or in chickens.
- Metabarcoding detected *Campylobacter* from samples at earlier timepoints than cultural isolation, including detection of ST6964 and ST257 from shed samples following clean-out and prior to the new flock placement. This is again consistent with carry-over from the previous flock contributing to infection of the study flock. However, metabarcoding does not differentiate between live and dead cells, and it is possible that the *Campylobacter* detected were not viable.
- The same STs detected by metabarcoding from the previous flock and following cleanout were also detected in cloacal samples from chickens at 10 days old, whereas no *Campylobacter* was isolated from cloacal swabs or caeca until chickens were 35 days old. However, different STs (STnd, no database ST designation) predominated in metabarcoding data from caecal and cloacal samples from 15 to 30 days; metabarcoding was not conducted on later samples.
- A surprising finding was the detection by metabarcoding of ST6964 and ST257 in samples from the breeder flock at the time of hatching of the study flock. Because only *C. lari* was isolated from these samples, they were not considered to be a contributing source for contamination of the study flock.
- Consistent with a high *Campylobacter* prevalence and wide range of STs isolated from catcher samples, multiple STs were also observed by metabarcoding of these samples.

Results support the utility and complementarity of this metabarcoding approach alongside WGS-based approaches for future applications characterising the diversity and population structure of *Campylobacter* in experimental studies. Moreover, the modular approach used in

the development of the method could also be applied toward genus- or species-specific metabarcoding methods for other pathogenic bacteria such as *Salmonella* or *Listeria*.

Taken together, this study identifies key areas where the poultry industry might focus on-farm risk management practices to reduce colonisation of broiler flocks by *Campylobacter* spp.. The most important areas of focus include farm transmission routes such as carry-over from the previous flock, and secondarily, contamination from chicken catching crews and equipment. As highlighted by the literature review, transmission routes for *Campylobacter* contamination of flocks will vary to some degree by farm location, seasonality and housing system. Therefore, analogous longitudinal surveys on different broiler farms might identify additional relevant areas for interventions by the poultry industry, toward the goal of reducing the food safety risk for poultry consumers.