NATIONAL MICROBIOLOGICAL DATABASE POULTRY MONITORING FOR *CAMPYLOBACTER*: INVESTIGATION OF NOT DETECTED RINSATES

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SUMMARY

Since April 2007, a testing programme has been in place at New Zealand poultry primary processing plants to determine the *Campylobacter* spp. status of birds entering primary processing, and carcasses at the end of processing. The end of processing testing includes rinsing of carcasses taken after the immersion chiller, plating of a rinse subsample, and counting of *Campylobacter* colonies, if present.

In these tests, the technical procedure requires the use of 400 ml of diluent, of which 2 ml is spread onto 6 plates of CBA or mCCDA agar. Effectively, the absence of colonies indicates no colony forming units (cfu) in the 2 ml subsample, i.e. a detection limit of 200 cfu in the entire rinsate. Results are collated by the National Microbiological Database (NMD).

The objective of this project was to determine whether a proportion of "not detected" (ND) results in the NMD database represent positive but low count (<200 cfu) carcasses. Unused rinsates were obtained from two processing plants, and if found to be ND, the rinsates were enriched and plated to confirm the presence or absence of *Campylobacter*.

Overall, 23 rinsate samples reported as ND (<200 cfu) were tested; of these 8 (34.8%) were found to contain *C. jejuni* (7 from Plant A and 1 from Plant B). It is notable that of the 8 positives, all but one came from birds whose flock/cut caecal samples tested negative.

Of the 21 rinsate samples received from caecal positive flocks, only one had a ND (<200cfu) result, and this was found to be positive by enrichment.

Of the 30 rinsate samples from caecal negative flocks, 8 had sufficient numbers of *Campylobacter* to be counted, and of the remaining 22 ND samples, 7 were positive, and 15 negative.

Although only one ND rinsate sample was obtained from a caecal positive flock, it did test positive, illustrating that a proportion of such samples can be positive. The total number of positive results after enrichment amongst ND rinsate samples (34.8%) suggests that the relationship between positive flocks and positive carcasses is difficult to assess from positive (countable) results alone. A large number of countable results (8/30) and positive presence/absence tests (7/22) from ND rinsate samples were found for carcasses originating from caecal negative flocks. Although false negative caecal testing results may occur, this suggests that cross contamination is occurring within primary processing chains.

Although 34.8% of the ND rinsate samples in this study were positive, it is acknowledged that such samples (with up to 200 cfu per carcass) will contribute a very small part of the overall risk to human health from *Campylobacter* on poultry.

1 INTRODUCTION

Since April 2007, a testing programme has been in place at New Zealand poultry primary processing plants to determine the *Campylobacter* spp. status of birds entering primary processing, and carcasses at the end of processing.

The testing involves:

- Analysis of 10 pooled caecal samples from flocks/cuts entering processing for presence/absence of *Campylobacter* spp.; and,
- Rinsing of carcasses taken after the immersion chiller, plating of a rinse subsample, and counting of *Campylobacter* colonies, if present.

In the second of these tests, the technical procedure (http://www.nzfsa.govt.nz/animalproducts/legislation/notices/animal-material-product/nmd/schedule-1-technical-procedures-nmd-final.pdf) requires the use of 400 ml of diluent, of which 2 ml is spread onto 6 plates of CBA or mCCDA agar, which are incubated under conditions conducive to the growth of *Campylobacter*, prior to bacterial enumeration if colonies are present. Effectively, the absence of colonies indicates no colony forming units (cfu) in the 2 ml subsample, i.e. a detection limit of 200 cfu in the entire rinsate.

Results are collated by the National Microbiological Database (NMD).

The New Zealand *Campylobacter* in poultry quantitative risk model (Lake *et al.*, 2007) predicts that the prevalence of infected birds/cuts entering primary processing is linearly related to the prevalence of contaminated carcasses exiting the immersion chiller. On the basis of NMD results from April 2007 to early 2009, this relationship has been questioned by some. Although hygienic dressing and effective decontamination will also result in negative birds, it is possible that the actual relationship between prevalence at entry and exit is masked by the "not detected" (ND) results.

The objective of this project was to determine whether a proportion of ND rinsate results in the NMD database represent positive but low count (<200 cfu) carcasses.

2 METHOD

The cooperation of two primary processing plants, Plant A and Plant B, was arranged, so that that a portion of unused leftover rinsate from the 400 ml could be sent to ESR.

- Plant A: sampling period from 29 October – 10 November 2008
- Plant B: sampling period from 4 February – 4 March 2009

All sampling was conducted during periods when acidified sodium chlorite treatment was not being used.

The portion of the rinsate (100ml of approximately 350 ml, giving a theoretical detection limit of <3 cfu) was poured into a sterile pottle so that the pottle was filled without headspace. It was then couriered to ESR in Christchurch, with the intention that NMD

testing timelines were achieved i.e. testing commenced with 30 hours of the rinse being taken, and samples when received were at 10°C or less.

On receipt at ESR, the entire rinsate sample (100ml) was enriched in double strength Exeter broth at 42°C for 48 hours. After that period, if necessary (i.e. if there was a delay in the processing plant laboratory informing ESR of their NMD result), the broth was moved to a 37°C incubator. This temperature is better for long term stability of *Campylobacter*, and is used to maintain reference culture broths.

Following receipt of the NMD result from the processing plant laboratory, samples for which there was a positive *Campylobacter* count were discarded. For rinsates for which the result was ND, the broth was then streaked onto mCCDA plus Blood agar to determine if *Campylobacter* colonies were present. If colonies were detected then a representative isolate was tested by Polymerase Chain Reaction to confirm its identity.

3 RESULTS

The processing plants provided information on cut number, caecal presence/absence testing, and counts (if found). Results are presented in Table 1. All isolates from ND rinsates were confirmed as *C. jejuni* by PCR. An overview of rinsate sample sources and results is presented in Figure 1.

4 DISCUSSION

For logistic reasons, a number of rinsate samples from Plant B were received at temperatures (>10°C) or times after sampling (>30 hours) that exceeded NMD guidelines and so were discarded without enrichment.

Overall, 23 rinsate samples reported as ND (<200 cfu) were tested; of these 8 (34.8%) were found to contain *C. jejuni* (7 from Plant A and 1 from Plant B). It is notable that of the 8 positives, all but one came from birds whose flock/cut caecal samples tested negative.

Of the 21 rinsate samples received from caecal positive flocks, only one had a ND (<200cfu) result, and this was found to be positive by enrichment.

Of the 30 rinsate samples from caecal negative flocks, 8 had sufficient numbers of *Campylobacter* to be counted, and of the remaining 22 ND samples, 7 were positive, and 15 negative.

Although only one ND rinsate sample was obtained from a caecal positive flock, it did test positive, illustrating that a proportion of such samples can be positive. The total number of positive results after enrichment amongst ND rinsate samples (34.8%) suggests that the relationship between positive flocks and positive carcasses is difficult to assess from positive (countable) results alone. A large number of countable results (8/30) and positive presence/absence tests (7/22) from ND rinsate samples were found for carcasses originating from caecal negative flocks. Although false negative caecal testing results may occur, this suggests that cross contamination is occurring within primary processing chains.

Although 34.8% of the ND rinsate samples in this study were positive, it is acknowledged that such samples (with up to 200 cfu per carcass) will contribute a very small part of the overall risk to human health from *Campylobacter* on poultry.

Figure 1: Summary of rinsate sample sources and results

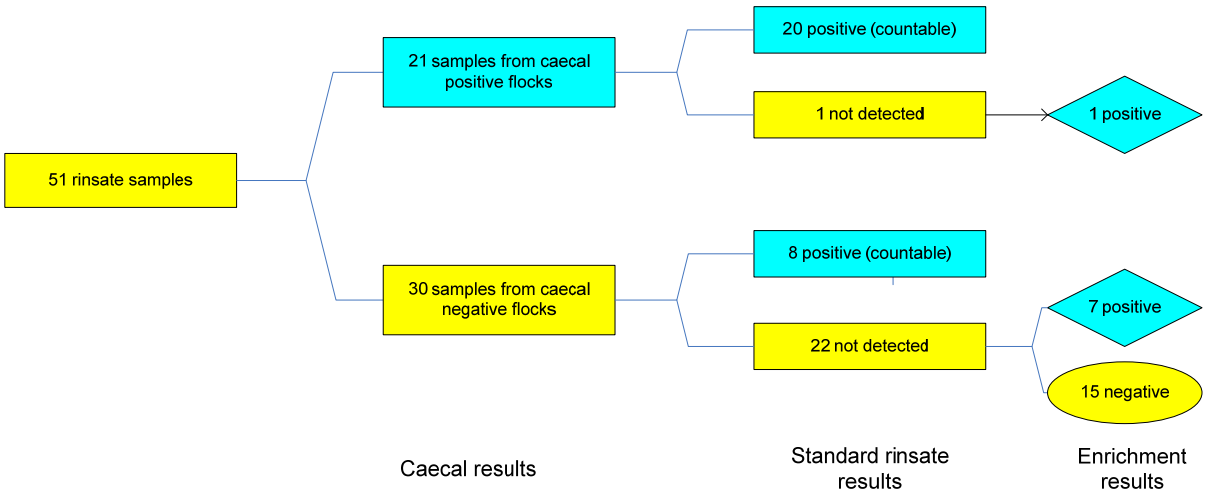

Table 1: Result of testing ND rinsates from Plants A and B

Date received	Cut	Caecal result	NMD Result	Presence/absence result
Plant A (2008)				
28 October	**1**	**Not detected**	**<200**	**Present**
28 October	**1**	**Not detected**	**200**	**Not tested**
28 October	**3**	**Not detected**	**400**	**Not tested**
29 October	**1**	**Not detected**	**<200**	**Absent**
29 October	**2**	**Not detected**	**<200**	**Absent**
29 October	**3**	**Not detected**	**<200**	**Absent**
30 October	**1**	**Not detected**	**<200**	**Absent**
30 October	**4**	**Not detected**	**<200**	**Present**
30 October	**2**	**Not detected**	**<200**	**Absent**
31 October	**1**	**Not detected**	**200**	**Not tested**
31 October	**2**	**Detected**	**2600**	**Not tested**
31 October	**2**	**Detected**	**44000**	**Not tested**
3 November	**2**	**Not detected**	**<200**	**Present**
3 November	**2**	**Not detected**	**<200**	**Present**
3 November	**3**	**Not detected**	**87400**	**Not tested**
4 November	**2**	**Not detected**	**<200**	**Absent**
4 November	**2**	**Detected**	**2400**	**Not tested**
4 November	**1**	**Detected**	**9600**	**Not tested**
5 November	**2**	**Detected**	**7400**	**Not tested**
5 November	**2**	**Not detected**	**<200**	**Present**
5 November	**2**	**Not detected**	**<200**	**Present**

6 November	**1**	**Not detected**	**400**	**Not tested**
6 November	**4**	**Not detected**	**2400**	**Not tested**
6 November	**1**	**Not detected**	**200**	**Not tested**
7 November	**3**	**Detected**	**1600**	**Not tested**
7 November	**3**	**Detected**	**5400**	**Not tested**
7 November	**1**	**Not detected**	**4000**	**Not tested**
10 November	**2**	**Not detected**	**<200**	**Absent**
10 November	**2**	**Not detected**	**<200**	**Absent**
10 November	**2**	**Detected**	**<200**	**Present**
Plant B (2009)				
4 February	**1**	**Not detected**	**<200**	**Absent**
4 February	**1**	**Not detected**	**<200**	**Absent**
4 February	**2**	**Detected**	**1200**	**Present***
4 February	**1**	**Not detected**	**<200**	**Absent**
10 February	**3**	**Detected**	**200**	**Not tested**
10 February	**3**	**Detected**	**200**	**Not tested**
12 February	**1**	**Detected**	**800**	**Not tested**
12 February	**1**	**Detected**	**1800**	**Not tested**
12 February	**1**	**Detected**	**400**	**Not tested**
13 February	**1**	**Not detected**	**<200**	**Absent**
13 February	**2**	**Detected**	**2000**	**Not tested**
13 February	**4**	**Detected**	**400**	**Not tested**
16 February	**1**	**Not detected**	**<200**	**Absent**
16 February	**1**	**Not detected**	**<200**	**Absent**
16 February	**1**	**Not detected**	**<200**	**Absent**
16 February	**3**	**Detected**	**400**	**Not tested**
16 February	**3**	**Detected**	**1600**	**Not tested**
19 February	**3**	**Detected**	**49800**	**Not tested**
24 February	**3**	**Detected**	**2200**	**Not tested**
24 February	**3**	**Detected**	**200**	**Not tested**
4 March	**1**	**Not detected**	**<200**	**Present**

*Initial result reported to ESR was that this sample was from a Not detected caecal flock, with a NMD result of <200. Hence it was tested by ESR. The NMD result was corrected later, and this result is correctly assigned in the discussion.

5 REFERENCES

Lake R, Hudson JA, Cressey P, Bayne G.. (2007) Quantitative risk model: *Campylobacter* spp. in the poultry food chain. Client Report FW0520. ESR: Christchurch Science Centre.