



***Campylobacter* spp. enumerated  
from drips trapped in leak-proof  
packaged retail poultry**

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***Campylobacter* spp. enumerated from drips trapped  
in leak-proof packaged retail poultry**

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## SUMMARY

The poultry industry and supermarkets in New Zealand have recently introduced leak-proof packaging for retail sale of whole birds and a proportion of packs of portions. This is intended to eliminate the risk of leaking drip fluid from these products contaminating the retail environment, and provided the products are properly handled by consumers, provides the potential for preventing cross contamination in the home. This study had the aim of providing basic data on the amount of drip retained within this type of packaging, and the numbers of *Campylobacter* spp. in the liquid.

Retail packs of leak-proof packaged poultry products were sampled in Auckland and Christchurch over a four week period in October 2007, to evaluate the amount of drip trapped in each pack and count of *Campylobacter*. The products included thirty whole birds from three brands, twenty five trays of chicken portions packed in leak-proof packaging (five samples each of skinless breasts, skin-on breasts, thighs, drums and nibbles) and five pottles of chicken livers. The volume of drip recovered from the leaked-proof packaged whole chickens ranged from 1.6 ml to 106.4 ml. Twenty five of the drips obtained from whole birds were positive for *Campylobacter*. *Campylobacter coli* was identified from one bird; all other isolates were identified as *C. jejuni*.

*Campylobacter* counts in drip from whole birds found to be positive ranged from  $<0.30 - 4.26$  Log<sub>10</sub> CFU/total drip. The *Campylobacter* counts present in the drips were independent of the volume of drips. More drip did not necessarily mean higher counts of *Campylobacter*.

The volume of drip extracted from the 25 portion trays when placed on a horizontal position (Drip A) ranged from 0 ml to 6.28 ml. Five trays of skin-on breast portions contained the most drip (mean 2.99 ml) followed by the five samples of skinless breast portions (mean 1.43 ml). The thigh portions had very little drip with only a mean volume of 0.31 ml per tray recovered. Trays containing drum and nibble portions had the least amount of drip; no drip was recovered from 7/10 samples when placed on the horizontal position, and drip volume extracted from the other three samples measured  $<0.5$  ml per sample.

In a second extraction of drip after trays of portions were placed in an inclined position (Drip B), further drip was recovered from only eleven samples. Two drum and two nibble samples

which did not produce drip in the horizontal position produced small amounts of drip (<0.3 ml of Drip B) when placed in the tilted position. Trays of skin-on breast portions produced the most amount of secondary drip.

Drip from 13 of the 25 trays of portions was positive for *Campylobacter*. Total (Drip A + Drip B) *Campylobacter* counts of these positive samples ranged from <4 - 109 CFU/total drip. All *Campylobacter* isolates were identified by PCR as *C. jejuni*.

The volumes of drip recovered from the pottles of livers were not as much as in leak-proof bags of whole birds but were more than those measured from trays of portions. The *Campylobacter* counts from the drips of liver samples ranged from 3.0 - >5.38 Log<sub>10</sub> CFU/total drip, the latter being the most contaminated sample containing more than 2.50 x 10<sup>5</sup> CFU/total drip of *C. jejuni*.

These data show that high numbers of *Campylobacter* are contained in the drip from some samples and highlight the importance of effective leak proof packaging by producers, and careful handling by consumers.



## 1 INTRODUCTION

A number of surveys have examined the prevalence and counts of *Campylobacter* in retail poultry in New Zealand (Hudson et al., 1999; Devane et al., 2005). The largest survey was undertaken from August 2003 to June 2004, and showed a high prevalence of 89.1% of *Campylobacter* in retail minced or diced chicken samples (Wong et al., 2007).

*Campylobacter* contamination on the outer surfaces of poultry packs has also been studied. A survey in early 2002 found *Campylobacter* on the surface of 72/300 (24%) packaged raw poultry products (whole chickens, portions, and offal) purchased from retail outlets in Christchurch (Wong et al., 2004). Thirty-two samples were contaminated with *Campylobacter* counts of <6 MPN per pack, while the remaining 40 samples were contaminated with counts ranging from 6 to >2200 MPN per pack. Packaged offal was more likely to be externally contaminated than packaged poultry meat, both with regard to the proportion of positive packs (52%) and higher counts of *Campylobacter* on positive packs.

During this survey it was noted that 121/300 (40%) of packs had liquid drip visibly leaking from the packs (Wong et al., 2004). Whole birds (68%) and offal trays (66%) were more likely to have leakage than portion trays (27%). The liquid drip is mainly chicken juice that has exuded from chicken meat after water is absorbed as a result of normal processing practices.

Such leakage was of concern because drips from these retail packs could cross contaminate the hands of customers and retail check out staff, check out counters, refrigerated display units and possibly other food products put next to poultry packs in shopping trolleys or baskets by customers. Cross contamination could also occur in the home, for example if leaky trays of poultry portions are stored in the upper shelves of domestic refrigerators.

Since the outer packaging study in 2002, the poultry industry and one supermarket franchise have introduced specific measures to control leakage of drip from poultry products. Whole chickens are now either double bagged and heat sealed, or placed into a heavier gauge plastic bag with an absorbent pad inserted into the bag and the opening clipped tightly to prevent drip from leaking out of the packaging. These leak-proof

packaged whole birds are supplied by the processors to the retail sector in both islands of New Zealand.

Retail packaging of chicken portions on trays has also been addressed by one of the grocery franchises, who have introduced leak-proof packaging of poultry products to its chain of supermarkets in the North Island. The chicken portions are packed on Plix™ trays (polystyrene trays with surface drainage holes into a sandwiched porous matrix) in a secondary packaging plant in Auckland. These trays of poultry products are double wrapped in a heavier gauge plastic film, heat sealed and then shrunk by hot immersion for presentation purposes during distribution and retail sales. These leak-proof packaged portions are not available to supermarkets associated with the franchise in the South Island. The remaining supermarkets that belong to other franchises in the North Island and all of the South Island still use the older procedure of packing chicken portions on Plix™ trays wrapped in cling film for retail sale.

While leak-proof packaging of poultry will not remove *Campylobacter* and/or *Salmonella*, if present, from the raw meat, it will ensure that these pathogens in the drip are contained within the package and will not cross-contaminate other products during storage/retail presentation, or hands of purchasers and check out counter staff. This packaging will also prevent cross contamination during storage in the home. Provided the packaging is handled carefully, it will also allow consumers to safely sequester and dispose of the drip, during preparation of the poultry for cooking. Any residual risk would result from careless handling, allowing drip to escape and potentially cross contaminate food preparation surfaces, utensils, or ready-to-eat foods.

Fundamental data on the amount of liquid drip in such packaging and the numbers of *Campylobacter* in the liquid are lacking. This project aimed to establish the volumes of drip in leak-proof packaged poultry, and to quantify the number of *Campylobacter*, if present, in the drip. The findings would be used to assist risk assessment of *Campylobacter* in the food chain.

## 2 MATERIALS AND METHODS

### 2.1 *Sampling*

A total of 30 whole birds, 25 samples of portions (in trays) and 5 samples of livers (in pottles) were purchased from supermarkets or other outlets on 5 shopping excursions (over 4 weeks in October 2007). Each week six whole chickens of difference sizes, two each of Brand A, Brand B and Brand C in leak-proof packaging, were purchased, either in Auckland or Christchurch, depending on availability. Portion trays (one each of skin-on chicken breast, skinless chicken breast, thighs, drums, and nibbles in leak-proof packaging) were purchased from one group of supermarkets in Auckland. Both types of breast portions and thighs consisted of 3 to 4 pieces per tray, drum trays consisting of 5 to 8 pieces, and medium size trays of nibbles, were sampled. Chicken livers were available only in lidded leak-proof plastic pottles and were purchased from supermarkets in Auckland.

Samples that were purchased in Christchurch were kept refrigerated until the next day and tested within 24 h of purchase. Samples purchased in Auckland were kept chilled (<5°C) and shipped to Christchurch by overnight courier and tested within 24 h of purchase.

### 2.2 *Extraction of drip from the whole birds*

Prior to opening and extraction of drip, each package was examined to check for signs of leakage to the outside of the packaging. The corner of the leak-proof bag was wiped with 70% alcohol to disinfect the surface. The corner was cut with a pair of sterile scissors and the drip was allowed to drain from the bag into a pre-weighed Whirlpak bag positioned on top of a balance. The chicken was allowed to drain freely for 2 minutes with slight shifting of the chicken position inside the bag but without squeezing of any absorbent pad that might have been placed inside the bag. The weight of the drip was recorded.

Enumeration of *Campylobacter* spp. was performed on the drip by following the procedures as described below. Total *Campylobacter* in the volume of drip collected was calculated and expressed as CFU/total drip.

### **2.3 Extraction of drip from the trays of chicken portions**

Two measurements of drip volume were made: (A) liquid available when the tray of portions was placed horizontally on a flat bench top (Drip A) and (B) trapped drip obtained after the tray of portions was inclined at an angle of about 60° for 30 min at 4°C to drain the trapped drip from the meat and from the inside of the internal matrix of the porous polystyrene Plix™ trays, to one end of the tray (Drip B). Leakage to the outside of the packaging was also noted if it occurred.

The volume of Drip A was measured by inserting a sterile disposable transfer pipette through a small hole on the plastic wrap which was wiped with 70% alcohol before puncturing. Visible drip was extracted from the surface of the tray and from the edges and beneath the chicken portions. Several holes were made to target different areas of the tray. Drip was collected in a pre-weighed Whirlpak bag and the weight recorded (The weight was taken as the volume of drip, assuming that 1 ml equals 1 g).

The volume of Drip B was measured after the tray had been tilted and drained, as described above. A corner at the lower end of the portion tray was wiped with 70% alcohol to sanitize the surface, and a sterile scalpel (flamed in alcohol) was used to make a nick at the sanitized corner of the tray. The drip was allowed to drain over two minutes into the opening of a pre-weighed Whirlpak bag. Shifting of the portions beneath the plastic film ensured that drip trapped underneath the portions was released from surface tension and allowed to drain into the bag. Enumeration of *Campylobacter* was in accordance to the procedure described below. Total *Campylobacter* in the volume of drip collected was calculated and expressed as CFU/total drip

### **2.4 Extraction of drip from chicken livers in leak-proof pottles**

The lid of the pottle was removed and the drip extracted with a sterile disposable transfer pipette from inside the pottle and around and under the pieces of liver for 2 min. The drip was collected in a pre-weighed Whirlpak bag. Enumeration of *Campylobacter* spp. was in accordance to the procedure described below. Total *Campylobacter* in the volume of drip collected was calculated and expressed as CFU/total drip

## 2.5 Enumeration of *Campylobacter* spp from drips

One ml of drip was spread over 3 mCCDA plate (pre-dried). Two further 0.1 ml volumes were spread over two mCCDA plates. The drip was further diluted by 1/10 by adding 1 ml of drip to 9 ml BPW ( $10^{-1}$  dilution) and two 0.1 ml volumes spread over two mCCDA plates. The remaining volume of drip was weighed and diluted by nine times in weight with Exeter broth. This enrichment and all mCCDA plates were incubated in microaerobic conditions at 42°C for 48 h.

Presumptive *Campylobacter* colonies on mCCDA plates were counted and five representative colonies were picked for PCR Identification. If characteristic *Campylobacter* colonies were present on the mCCDA plates, the enrichment culture was not streaked since a count was made from the mCCDA plates. However if no *Campylobacter* colony was present on the mCCDA plates, a loopful from the enrichment culture was streaked onto an mCCDA plate to isolate *Campylobacter* from the enrichment broth. The plate was incubated microaerobically 42°C for 48 h. Presence of *Campylobacter*-like colonies was followed by selecting 5 isolates for PCR confirmation.

The number of *Campylobacter* spp. present per ml of drip was used to calculate the total *Campylobacter* load in the whole recovered volume of drip (expressed as CFU/total drip) by extrapolation as follows:

The count (CFU) over 3 mCCDA plates were multiplied by the volume of drip and expressed as CFU/total drip.

If the 0.1 ml fraction was used to enumerate the count, then the average count of the duplicate plates were calculated, multiplied by a factor of 10 followed by multiplication by the volume of the drip (results expressed as CFU/total drip).

For volumes which were less than 1 ml, the whole volume was spread onto one or two plates depending on the amount of drip present. The count was presented as CFU/total drip.

For counts <1 CFU/ml and where no colonies were isolated from the spread plates but present in the presence/absence testing of the remaining drip, the counts were expressed as less than the volume of the drip recovered from the tray (for example if 5 ml of drip was recovered and *Campylobacter* was isolated only from the presence/absence test of the remaining drip after plates were spread, then the count would be expressed as <5 CFU/total drip).

### 3 RESULTS

#### 3.1 *Drip volume and Campylobacter spp. counts from leak-proof packaged whole birds*

Thirty whole chickens in leak-proof packaging comprising ten birds from each Brand, were purchased from retail outlets in Auckland and Christchurch. The volume of drip recovered from the leaked proof packaged whole chickens ranged from 1.6 ml to 106.4 ml (Table 1). Brand A chicken packs contained the most amount of drip trapped inside the leaked proof bags (mean volume 68.1 ml, range 30.6 – 106.4 ml) followed by Brand C chicken packs (mean volume 36.2 ml, range 1.6 – 88.0 ml) and Brand B chicken packs (23.4 ml, range 2.3 – 52.5 ml). Two packs from Brand A and three packs from Brand C showed signs of leakage despite of the leak-proof packaging. Leaks occurred when the heat sealed seams were poorly sealed together.

The *Campylobacter* status of drips recovered from each brand of poultry showed that 9/10 drips from Brand A were positive compared with 8/10 each from Brand B and Brand C (Table 1). *Campylobacter coli* was identified from one bird of Brand B and all other isolates were identified as *C. jejuni*.

For seven of the 25 positive drip samples, the counts were below the limit of detection by the spread plate method (1 CFU/ml being the limit of detection), but the enrichment of the remaining drips were positive for *Campylobacter*. These counts were expressed as less than (<) the volume of the drip. Altogether, 12/30 (40%) samples of drip from whole birds had either no *Campylobacter* detected or counts were below the limit of detection.

To compare between brands, means and ranges were calculated for the highest 5 counts (the top 5 were chosen since counts were available for comparison for all brands). Brands A and C were very similar: Brand A: mean 3.79 Log<sub>10</sub> CFU/total drip (range 2.27 – 4.08 Log<sub>10</sub> CFU/total drip); Brand C: mean 3.75 Log<sub>10</sub> CFU/total drip (range 2.60 – 4.26 Log<sub>10</sub>CFU/total drip). The mean count for Brand B was lower at 2.68 Log<sub>10</sub> CFU/total drip (range 1.71 – 3.21 Log<sub>10</sub> CFU/total drip), but this very limited dataset does not suggest major differences between brands.

The *Campylobacter* counts present in the drips were independent of the volume of drips (Fig. 1). A higher volume of drip did not necessarily mean higher counts of *Campylobacter*.

**Table 1. Count of *Campylobacter* spp. and volume of drip trapped in leak-proof packaged retail whole broiler chickens**

Brand A*			Brand B			Brand C†		
Total volume of drip (ml)	Status	Count in drip (Log <sub>10</sub> CFU)	Total volume of drip (ml)	Status	Count in drip (Log <sub>10</sub> CFU)	Total volume of drip (ml)	Status	Count in drip (Log <sub>10</sub> CFU)
70.5	Positive	4.08	14.3	Positive‡	3.21	20.2	Positive	4.26
46.9	Positive	4.03	23.0	Positive	2.79	22.8	Positive	3.78
76.2	Positive	3.68	25.9	Positive	1.71	29.9	Positive	3.36
106.4	Positive	3.44	52.5	Positive	1.72	21.6	Positive	3.11
46.5	Positive	2.27	25.6	Positive	1.71	79.1	Positive	2.60
77.0	Positive	1.86	33.9	Positive	<1.53	88.0	Positive	2.42
73.1	Positive	<1.89	12.4	Positive	<1.08	1.6	Positive	2.34
62.7	Positive	<1.80	2.3	Positive	<0.30	42.7	Positive	<1.63
30.6	Positive	<1.49	7.1	Negative	0	26.6	Negative	0
91.2	Negative	0	36.8	Negative	0	30.0	Negative	0
Mean 68.1			Mean 23.4			Mean 36.2		

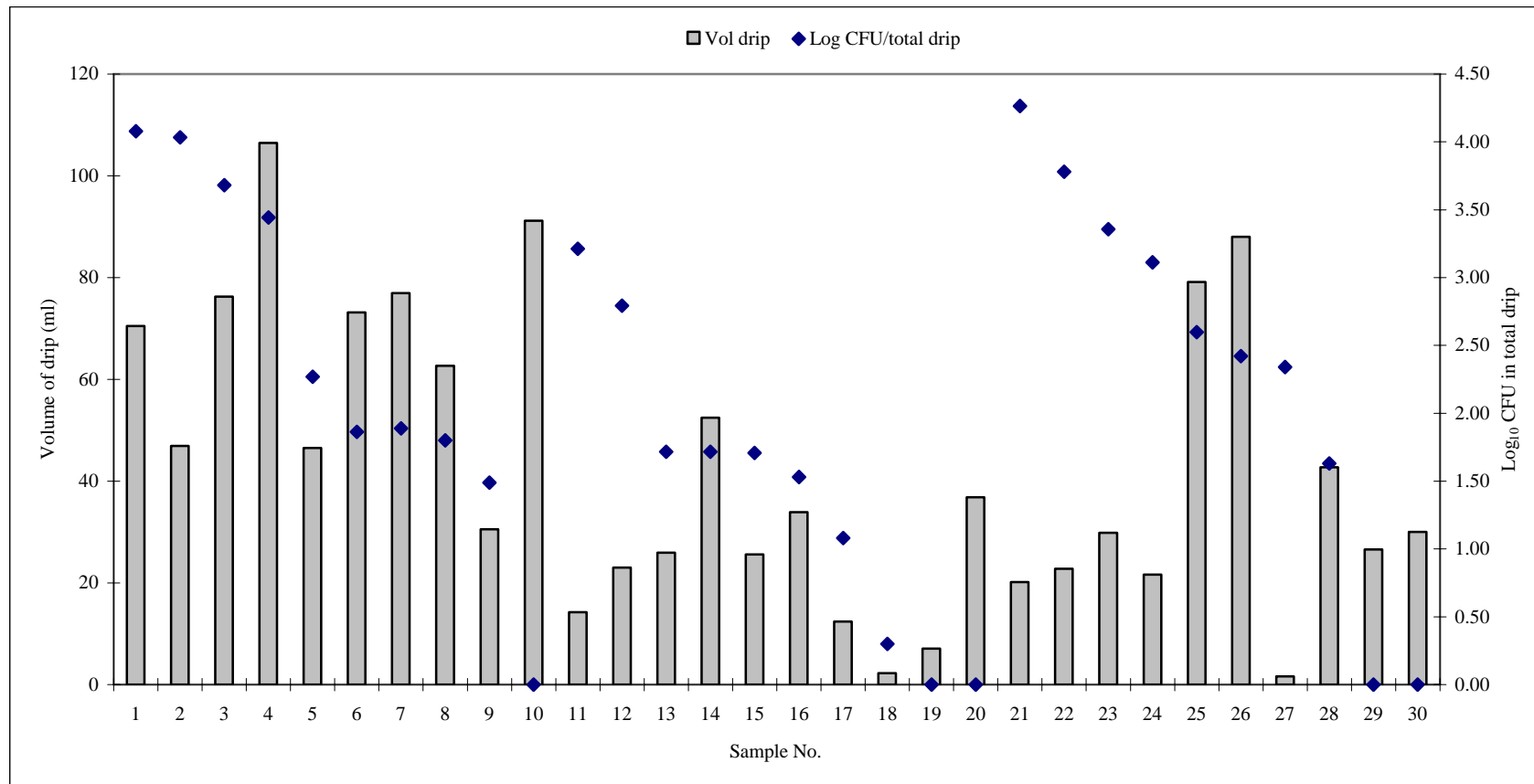
\* Two packs showed leakage despite of the leak-proof packaging.

† Three packs leaked despite of the leak-proof packaging.

‡ *Campylobacter* identified by PCR as *C. coli*. All other positive samples were identified as *C. jejuni* by PCR.



**Figure 1.** *Campylobacter jejuni* counts in chicken drips collected from leak-proof whole birds



Samples 1-10 (Brand A); samples 11-20 (Brand B); samples 21-30 (Brand C).

### 3.2 *Drip volume and Campylobacter counts from leak-proof packaged chicken portions*

Twenty five samples of chicken portions comprising of five samples each of skinless breasts, skin-on breasts, thighs, drums and nibbles, were purchased from supermarkets in Auckland over five weeks. All the chicken portions purchased were packed on Plix™ polystyrene trays and sealed in leak-proof packaging.

The volume of drip extracted from the portion trays while positioned horizontally on a bench (Drip A), was small (range 0 - 6.28 ml per tray) (Table 2). Trays of breast portions contained the most drip: skin-on mean 2.99 ml, skin-off mean 1.43 ml. The thigh portions had very little drip: mean 0.31 ml. Trays containing drum and nibble portions had the least amount of drip; no drip was recovered from 7/10 samples and the other three samples measured <0.5 ml per sample.

Measurable amounts of further drip (Drip B) was recovered from only eleven samples (Table 2). Two drum and two nibble samples which did not produce drip in the horizontal position produced small amounts of drip (<0.3 ml of Drip B) when placed in the tilted position. Overall, trays of skin-on breast portions produced the highest amount of secondary drip.

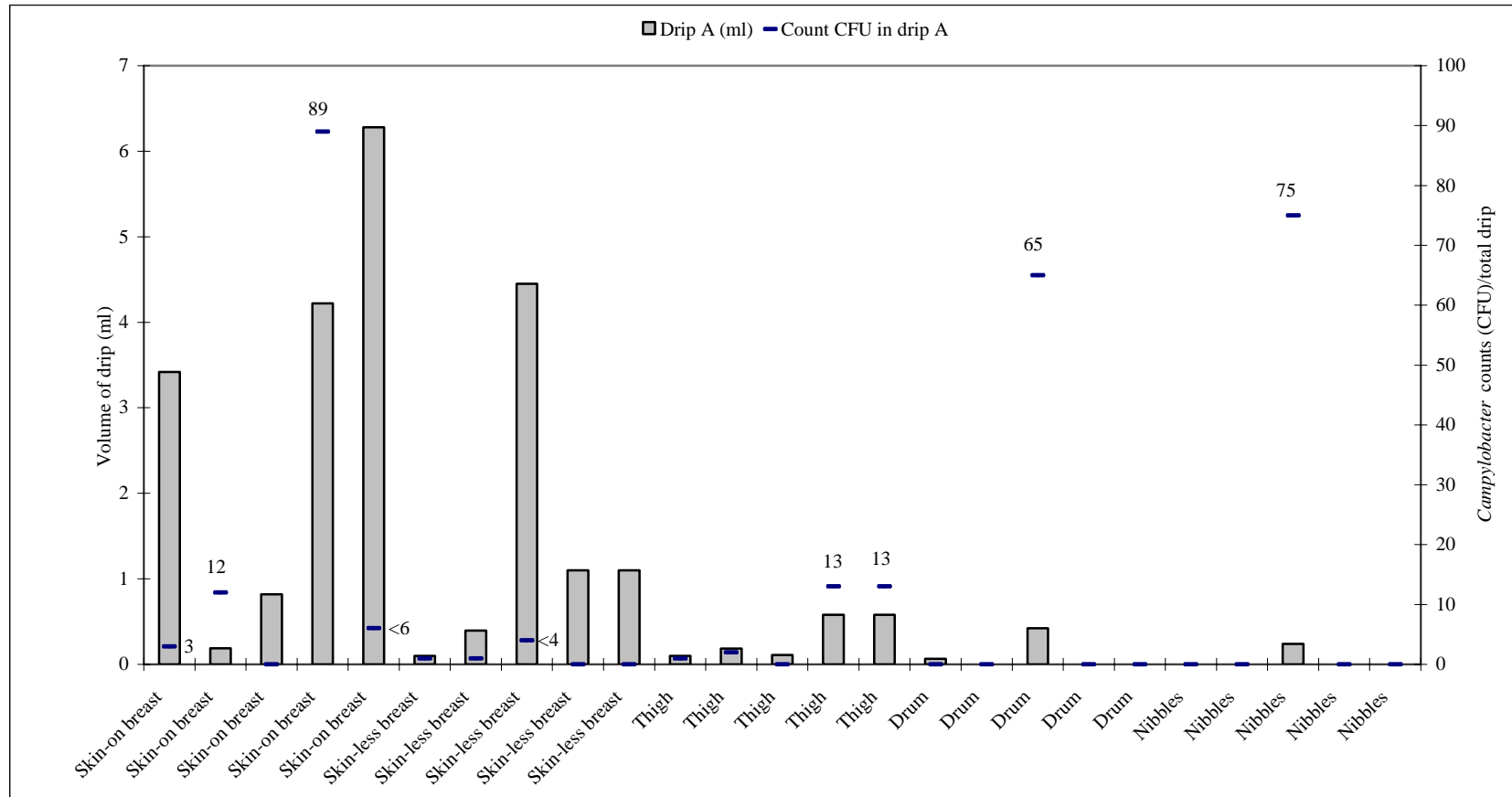
Enumeration of *Campylobacter* spp. from the drips extracted from trays placed on a horizontal position (Drip A) gave thirteen positive results (Fig. 2), of which seven were below the countable limit of <10 CFU/total drip. All *Campylobacter* isolates were identified by PCR as *C. jejuni*.

Five drip B samples were positive, all of which were amongst the 13 positive samples detected from Drip A. Combining the results from Drip A with that of Drip B only resulted in a slight increase in *Campylobacter* count in three of the positive samples, while the remaining two positive Drip B samples did not have measurable counts, but changed the “less than” result for these trays (Fig. 3).

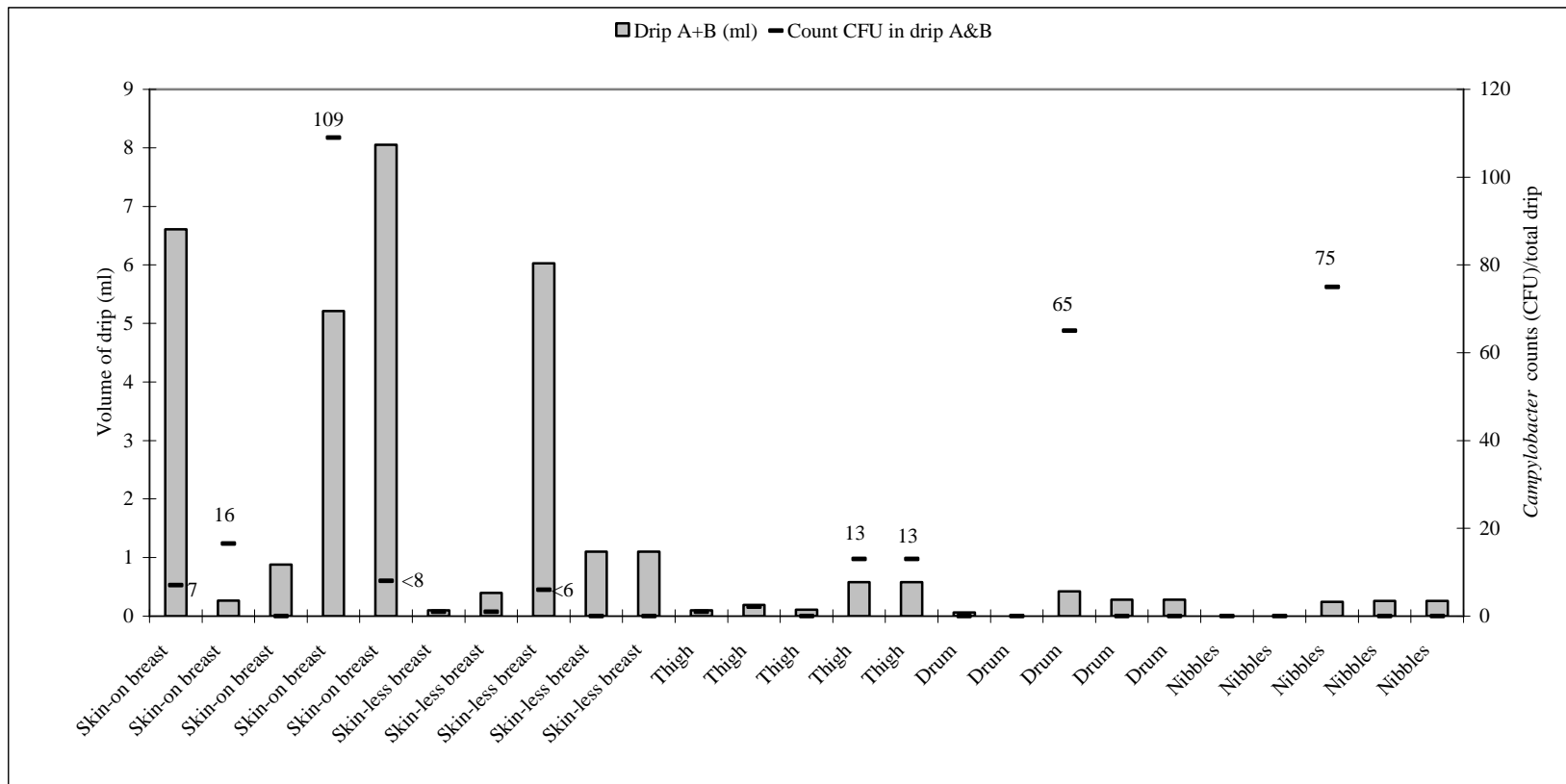
**Table 2. Volume of drip extracted from trays of leak-proof packaged chicken portions**

Tray Sample No.	Skin-on breasts		Skinless breasts		Thighs		Drum		Nibbles	
	Drip A (ml)	Drip B (ml)	Drip A (ml)	Drip B (ml)	Drip A (ml)	Drip B (ml)	Drip A (ml)	Drip B (ml)	Drip A (ml)	Drip B (ml)
1	3.42	3.19	0.10	0	0.10	0	0.07	0	0	0
2	0.19	0.071	0.40	0	0.19	0	0	0	0	0
3	0.82	0.06	4.45	1.58	0.11	0	0.42	0	0.24	0
4	4.22	0.99	1.1	0	0.58	0	0	0.28	0	0.26
5	6.28	1.77	1.1	0	0.58	0	0	0.28	0	0.26
Mean	2.99	1.22	1.43	0.32	0.31	0.00	0.10	0.11	0.05	0.10

**Figure 2.** *Campylobacter* counts and volume of drip from portion packs while the packs were placed horizontally on bench top (Drip A)



**Figure 3. Total *Campylobacter* counts and total volume of drip (Drip A and B) retrieved from portion packs while placed horizontally followed by draining after being kept at a 60° angle in a tilted position**



### 3.3 Drips recovered from leak-proof pottles of chicken liver

Only five samples of liver were purchased over the five week period, all were packaged in tight-lidded plastic pottles. The volumes of drip recovered from the pottles of livers (Table 3) were inbetween those for whole birds (Table 1) and leak-proof packaged trays of portions (Table 2). All five drip samples were positive and the total *Campylobacter* counts (Table 3) were high with one sample exceeding 5.38 Log<sub>10</sub> CFU/total drip.

**Table 3. Volume of drip and *Campylobacter* counts from pottles of chicken liver**

Sample No.	Volume of drip extracted (ml)	Count (Log <sub>10</sub> CFU)/ml of drip	Status	Total count (Log <sub>10</sub> CFU/total drip)
1	14.12	3.55	Positive	4.70
2	5.14	3.20	Positive*	3.92
3	8.36	2.08	Positive	3.00
4	6.60	2.75	Positive	3.57
5	9.67	>4.40	Positive	>5.38

\* Except for this sample where *C. jejuni* and *C. coli* were isolated, all other samples only isolated *C. jejuni*, as identified by PCR.

## 4 DISCUSSION

Most quantitative risk assessments for *Campylobacter* on poultry have modelled the scenario of transfer of bacteria from the product to surfaces or utensils in the food preparation area, and thence to other foods or back to the cooked chicken resulting in consumer ingestion. These models have usually been based on contact between the surface of a poultry portion or carcass, and the environmental surface, with transfer of a proportion of the total *Campylobacter* numbers. However, a Canadian model from 2000 was based on estimations of loosely adhered *Campylobacter* being in drip fluid, with consumers ingesting 0.5 – 1.5 ml of drip through cross contamination (WHO/FAO, 2002).

Estimation of the risk from drip fluid would need information on the handling practices of food preparers that is not available at present. Appropriate handling and disposal of liquid drip when preparing poultry should result in no cross contamination from this source. It is not known what proportion of the total *Campylobacter* numbers present on the poultry products in this study are represented by the counts from the drip. However, based on the predicted distribution for *Campylobacter* numbers on whole carcasses exiting the immersion chiller from the New Zealand quantitative risk model (mean 2.871 Log<sub>10</sub> CFU) (Lake et al., 2007) the proportion in the drip fluid could easily be a high proportion of the total. This highlights the importance of proper handling of the drip in domestic and commercial kitchens.

In comparison to the drip volume recovered from the whole birds, the amount of drip collected from the leak-proof packaged portion trays was much lower (mean volume, range 0.05 ml – 2.99 ml). Overall, the trays of skin-on breast portions contained the most drip while a tray of skinless breast portions carried the most drip of all the portion trays. The *Campylobacter* counts obtained from the drips of 25 portions trays of various portions were also lower than for whole carcasses, the top three counts being < 2 Log<sub>10</sub> CFU/total drip.

While the Plix™ trays are designed to absorb excessive drip into their polystyrene matrix. The experiments were designed to capture this volume as a separate sample; however, the amount of drip trapped in the trays (Drip B) was small in volume. The most secondary

drip (Drip B) came from the skin-on breast portions but even then a mean of only 1.22 ml was recovered. This suggests that fluid retention by the trays was effective, or that portions are less wet when first packed into leak-proof packed trays.

In contrast, drip from pottles of chicken livers carried the overall highest load of *Campylobacter* spp. (3.00 - >5.38 Log<sub>10</sub> CFU/total drip) in a moderate amount of drip (5.14 – 14.12 ml). This indicates that proper handling and disposal of drip from these poultry products is particularly important to minimise any risk of cross contamination.

The observation that 5/30 of the whole bird samples showed evidence of leakage due to ineffective sealing of the packaging indicates that improvements in packaging are needed.



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