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# *Campylobacter* spp. in Icelandic poultry operations and human disease

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## SUMMARY

We describe the observed relationship of campylobacter in poultry operations to human cases in a closed environment. During 1999 in Iceland, domestic cases of campylobacteriosis reached peak levels at 116/100 000 and in 2000 dropped to 33/100 000. Approximately 62% of broiler carcass rinses were contaminated with *Campylobacter* spp. in 1999. During 2000, only 15% of the broiler flocks tested *Campylobacter* spp. positive. In 2000, carcasses from flocks which tested positive on the farms at 4 weeks of age were subsequently frozen prior to distribution. We suggest that public education, enhanced on-farm biological security measures, carcass freezing and other unidentified factors, such as variations in weather, contributed to the large reduction in poultry-borne campylobacteriosis. There is no immediate basis for assigning credit to any specific intervention. We continue to seek additional information to understand the decline in campylobacteriosis and to create a risk assessment model for *Campylobacter* spp. transmission through this well defined system.

## INTRODUCTION

Campylobacteriosis continues as the most frequent bacterial cause of human gastroenteritis in many Nordic countries [1, 2]. In the United States, approximately two million cases of human campylobacteriosis occur each year [3]. Researchers report that a majority of human campylobacteriosis is associated with poultry [3–10]. Although it is strongly suggested that poultry is the most important reservoir for *Campylobacter jejuni*, some disagreement exists with this assertion. Part of this lack of accord originates over disputed epidemiological assumptions that have been employed to extrapolate such assertions.

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Consumers throughout the United States access poultry products produced and distributed around the country. Although poultry and human *C. jejuni* isolates share serological similarities, irrefutable cause and effect evidence (i.e. causality and clonality of isolates) for the largest portion of cases has been difficult to demonstrate. Tracking strains throughout the United States or, even through a single poultry operation has proved a formidable task [11]. Therefore, irrefutable data describing transmission have been lacking. Additionally, the agricultural community (i.e. the poultry industry) observe that numbers of campylobacter per processed carcasses have been dramatically reduced over the past several years. The rate of campylobacteriosis in the United States as detected by 'FoodNet' at the five original surveillance sites in 1999 was estimated at 17·3 per 100 000, reduced from the 1996 estimate of 23·5 per 100 000 [12]. One important line of argument is that, if the public exposure has been dramatically reduced in this most significant source, why is there yet such a high frequency in the general population?

Iceland is a modern island nation, having human campylobacteriosis at frequencies equal to, or exceeding those reported in most other developed countries. Its limited size (a population of 277 184 in 1999, and 281 154 in 2000) and completely characterized poultry operation (national production of $\sim$2 500 000 broilers per year), provides a unique opportunity to characterize contributing factors leading to poultry colonization and transmission to humans. Iceland is self-sufficient in its broiler production and consumption. Intensive human disease surveillance gives rise to the potential to gather all domestic and foreign acquired isolates from human cases within that fixed ecosystem and the potential to determine the contributing sources which lead to campylobacteriosis. We have been conducting a comprehensive study gathering data with the intention of creating a risk assessment model applicable to Iceland and other industrialized countries. This article provides observations on the relationship between poultry contamination and human disease in Iceland.

## MATERIALS AND METHODS

### Poultry samples

Broiler carcass rinses during 9 August–26 October 1999 were obtained at the two commercial plants processing about 98% of the poultry in Iceland. At that time, these plants employed water chilling of the processed carcasses containing up to 50 p.p.m. chlorination in the makeup waters and had residual levels of 2–5 p.p.m. The smaller processing plant, Abattoir B, was more modern than Abattoir A. Abattoir B used modern equipment such as 'inside-outside' washers as part of its processing. Although the lines did not employ modern evisceration technology, there was good comparability to more modern systems. The lines ran at 800–1200 birds/h. Fully processed carcasses were sampled after chill tank cooling of the carcasses, using new sterile latex gloves for each processed bird. The sample was placed in a new Whirlpak plastic bag, 100 ml of sterile water added, and the carcasses were shaken for 1 min. The rinse suspensions were aseptically transferred to sterile 100 ml screw-capped centrifuge bottles and were immediately chilled to refrigeration temperatures. Samples were then tightly packaged into approved styrofoam containers to sustain chilled temperatures and the remaining unfilled spaces were occupied by centrifuge bottles containing chilled water. The bottoms and tops of the packages contained frozen artificial ice packets to maintain the desired $\sim$4 °C temperature. Weekly shipments were made overnight by courier service, clearing US Customs at the port of entry, and over 90% of the samples arrived at the Athens, Georgia laboratory still chilled ($<$5 °C) within 48 h of the bird processing. From May to the end of July 2000, same day sampling of caeca from processed flocks in our Reykjavik, Iceland laboratory (at the University of Iceland, Microbiology Laboratory) provided quantitative estimates of the organism. When broiler flocks were detected as positive by caecal or cloacal sample testing (described below), carcass weep fluids were aseptically drawn from 4 °C stored retail packaged poultry. During a period of low flock prevalence, additional sampling of live haul crates and carcass rinse were added to further enhance flock sampling sensitivity. Poultry production samples (grandparent breeders, sexually immature breeders and parent breeders) were also assessed during this year 2000 time frame. Faecal droppings and crate swabs were assayed by microbiological methods described below. All samples were individually gathered and maintained under chilled conditions. The grandparent breeders faecal droppings were aseptically gathered in Sweden and shipped overnight under chilled conditions to our Iceland laboratory for analysis. During 2000, poultry faeces, caecal specimens, carcass rinses and weep fluids were quantitatively estimated for the presence of *Campylobacter* spp. as

described below. Intestinal materials were collected in sterile stomacher bags, sterile screw-capped, 50 ml centrifuge tubes or in specimen cups, placed on ice and diluted 1 : 3 (w/v) with saline solution.

In addition to the above, the Chief Veterinary Office, following introduction of legislation in Iceland, conducted an official sampling programme, initiated on 30 January 2000, and continuing until the end of 2000 and thereafter. Included in this sampling programme were cloacal swabs taken from 10 birds per flock at 4 weeks of age, 10 cloacal swabs batched into one sample per flock at the processing plant and 50 neck skins (5 batched samples of 10 neck skins each) from carcasses after evisceration at the processing plant. This sampling programme was managed by the National Veterinary Office for Poultry Diseases, and all samples were analysed in the Laboratory of the Institute for Experimental Pathology, Keldur.

### Human case samples

Two clinical laboratories in Iceland receive all stool samples from humans for the isolation of *Campylobacter* spp. Specimens are received from health centres, private physicians, outpatient clinics and hospitals. Information on recent foreign travel was gathered from the physicians submitting the specimens or obtained directly from the patients. It is normal practice to culture stool samples from all patients admitted to hospital clinics with diarrhoea and from outpatients with severe and/or prolonged diarrhoea. Over 95% of the human samples are processed at the Microbiology Department, Landspitali University Hospital, where they are inoculated onto Skirrow's agar (Oxoid, Basingstoke, England) and incubated at 42 °C in a microaerobic atmosphere for 48 h (72 h from November 1999). Colonies with a typical macroscopic appearance, positive oxidase and catalase reactions and typical morphology and motility on microscopy and without reaction in API 10S (bioMérieux) are identified as *Campylobacter* spp. From November 1999 all samples were additionally inoculated onto CCDA (Charcoal cefoperazone deoxycholate) agar (Oxoid) at 37 °C for an 8 month period and all plates were incubated for 72 h. Furthermore, identification to the species level (hippurate hydrolysis, indoxyl acetate, nalidixic acid and cephalothin sensitivity and API Campy) and sensitivity testing for erythromycin and ciprofloxacin (E-test, AB Biodisk) are now performed. The bacteriology laboratory at Akureyri District Hospital also processes stool samples for *Campylobacter* spp., and samples are inoculated onto Skirrow's agar as outlined above. All presumptive *Campylobacter* spp. isolates are referred to the Landspitali University Hospital for confirmation and susceptibility testing where they are subsequently stored at −70 °C.

### Microbiological methodology for poultry and data recording

Materials and methods for analysis of poultry carcasses, intestinal materials and crate swabs were as previously described [13, 14]. Briefly, 0·1 ml of the diluted faecal or caecal sample was spread plated onto Campy-Cefex plates [15]. The samples were serially diluted in phosphate buffered saline, pH 7·2, and the dilutions plated onto the selective agar. The plates were incubated under a microaerobic gas atmosphere of 5% oxygen, 10% carbon dioxide and 85% nitrogen at 42 °C for 48 h; 0·1 ml of the carcass rinse or weep fluids were placed onto duplicate Campy-Cefex plates for quantitative analysis. Serial dilutions were made as described above. Plates were incubated under a microaerobic atmosphere at 42 °C for up to 48 h, when colony forming units were enumerated. Detection limits were estimated as 30 c.f.u./g of intestinal material. Estimates were converted into $\log_{10}$ values and averaged among all samples taken within each report lot. Microscopical examination for characteristic motility and morphology were considered presumptive detection of *Campylobacter* spp. and latex agglutination was used as confirmatory testing.

Regulatory samples were analysed by modified the NMKL (the Nordic Committee on Food Analysis) method no. 119, 2nd ed., 1990, *Campylobacter jejuni/coli* detection in Foods [16]. Neck skin (10 g) from processed poultry carcasses were aseptically placed in 100 ml Preston enrichment broth. Cloacal swabs were placed into tubes containing 9 ml of Preston enrichment broth. The enrichment cultures were incubated 48 h at 42 °C under a microaerobic atmosphere. The enrichment culture and stools (or caecal materials) were then transferred onto selective modified Charcoal Cefoperazone Deoxycholate agar (mCCD agar) and incubated 48 h at 42 °C under a microaerobic atmosphere. Subcultures of the isolates were confirmed by motility testing, Gram reaction, bacterial morphology, hippurate hydrolysis and indoxyl acetate hydrolysis.

## RESULTS

### 1999 poultry sampling

The poultry rinse samples obtained during August–October 1999 represented 1·45% of all the slaughtered birds processed at Abattoir A and 2·71% at Abattoir B. During this sampling, 29 of 36 broiler flocks sampled were produced on farms nos 1 and 2 and processed in Abattoir A (Table 1). These two farms produced multiple flocks having both high (100%), or comparatively low rates (42%) of *Campylobacter* spp. contaminated flocks as determined by the presence of the organism on the processed carcasses.

As recorded in Table 2, data from farm 1 (Abattoir A) operations yielded a consistently high frequency of processed product contamination throughout the 2·5 months of sampling. Fifteen of the 17 flocks sampled provided individual carcass contamination rates of 75% or higher. The other two flocks had contamination rates between 20 and 30%. The rates of carcass contamination seen on birds produced from farm 2 were considerably more variable than from farm 1 (Table 2). Only 3 of those 12 flocks tested had carcass contamination frequencies greater than 88% and, 8 of the 12 flocks had carcasses with frequencies equal to or less than 20%. There was no apparent variation in contaminated carcass rates over time. Highly contaminated flocks were detected both before and after flocks without detected contamination.

### Human cases of domestically acquired *Campylobacter* spp.

The domestically acquired cases of human campylobacteriosis during 1997–2000 was provided by Landspitali University Hospital, Reykjavik, Iceland, through the national Icelandic *Campylobacter* spp. surveillance and are presented in Figure 1. The case rate reported in 1997 was relatively low, with a total of only 38 domestic cases detected among the population of ~275 000 (13·8 cases/100 000). There was an increase in 1998 to 143 cases (52 cases/100 000). Campylobacteriosis detection peaked in 1999 with 326 cases (116 cases/100 000), and fell again in 2000 to 92 domestic cases (33 cases/100 000). In contrast to the domestic cases in 2000, the foreign acquired cases increased from 82 in 1999 to 130 cases. Foreign acquired cases showed a progressive increase during 1997–2000.

Figure 2 provides a week by week comparison for human cases of *Campylobacter* spp. in 1999 and 2000.

Table 1. *Broiler chicken flocks produced in Iceland and processed from 9 August to 26 October 1999. The frequency of detected* Campylobacter *spp. contamination on the processed carcasses is indicated*

| Location of production/ processing | Number of flocks assayed | Percentage of campylobacter positive flocks |
|---|---|---|
| Abattoir A | | |
| Farm 1 | 17 | 100 |
| Farm 2 | 12 | 42 |
| Farm 3 | 1 | 100 |
| Farm 4 | 1 | 0 |
| Farm 5 | 1 | 100 |
| Abattoir B | | |
| Four small private farms | 4 | 0 |

Table 2. *Frequency of* Campylobacter *spp. contamination on carcasses from broiler chicken flocks grown on farm 1 and farm 2 and processed in Abattoir A during 1999*

| Date (1999) | Farm 1 | Farm 2 |
|---|---|---|
| 9 Aug. | 75/75*(100†) [2]‡ | 24/25 (96) [1] |
| 16 Aug. | 18/75 (24) [2] | 0/25 (0) [1] |
| 23 Aug. | 68/75 (90) [1] | 1/25 (4) [2] |
| 24 Aug. | 20/25 (80) [1] | 10/50 (20) [2] |
| 30 Aug. | 57/75 (76) [2] | 0/25 (0) [1] |
| 31 Aug. | 67/75 (89) [1] | 2/25 (4) [2] |
| 7 Sep. | NA§ | 44/50 (88) [2] |
| 14 Sep. | 75/75 (100) [1] | NA |
| 20 Sep. | 73/75 (97·6) [1] | 0/25 (0) [2] |
| 21 Sep. | NA | 49/50 (98) [3] |
| 28 Sep. | 74/75 (98·6) [2] | 0/25 (0) [1] |
| 4 Oct. | 74/75 (98·6) [2] | 10/25 (40) [1] |
| 5 Oct. | 75/75 (100) [2] | 2/25 (8) [1] |
| 11 Oct. | 75/75 (100) [1] | NA |
| 12 Oct. | 100/100 (100) [1] | NA |
| 18 Oct. | 100/100 (100) [1] | NA |
| 19 Oct. | 50/50 (100) [1] | NA |
| 25 Oct. | 75/75 (100) [1] | NA |
| 26 Oct. | 28/99 (28·2) [1] | NA |

* Number of campylobacter positive samples per number of samples taken.
† % of campylobacter positive samples in parentheses.
‡ Figures in square brackets indicate order of slaughter group on day of sampling.
§ Samples not available (NA).

These data were provided by the State Epidemiologist of Iceland as a part of their monitoring responsibility. Although domestically acquired cases (Fig. 2*a*) were not eliminated, substantial reductions in campylobacteriosis were reported in 2000. There was still a

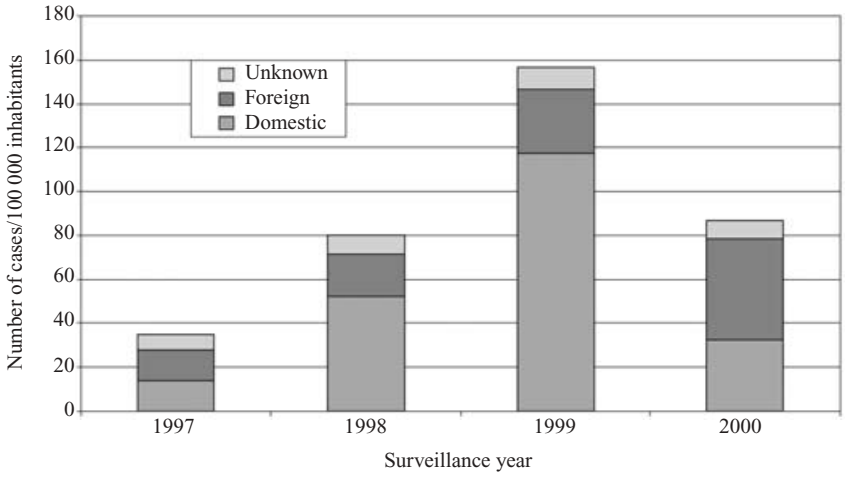


**Fig. 1.** Domestically and foreign acquired human infections of *Campylobacter* spp., 1997–2000. The national Icelandic surveillance is provided by the Landspitali University Hospital, Reykjavik. Positive stool specimens peaked in 1999 and fell in 2000.

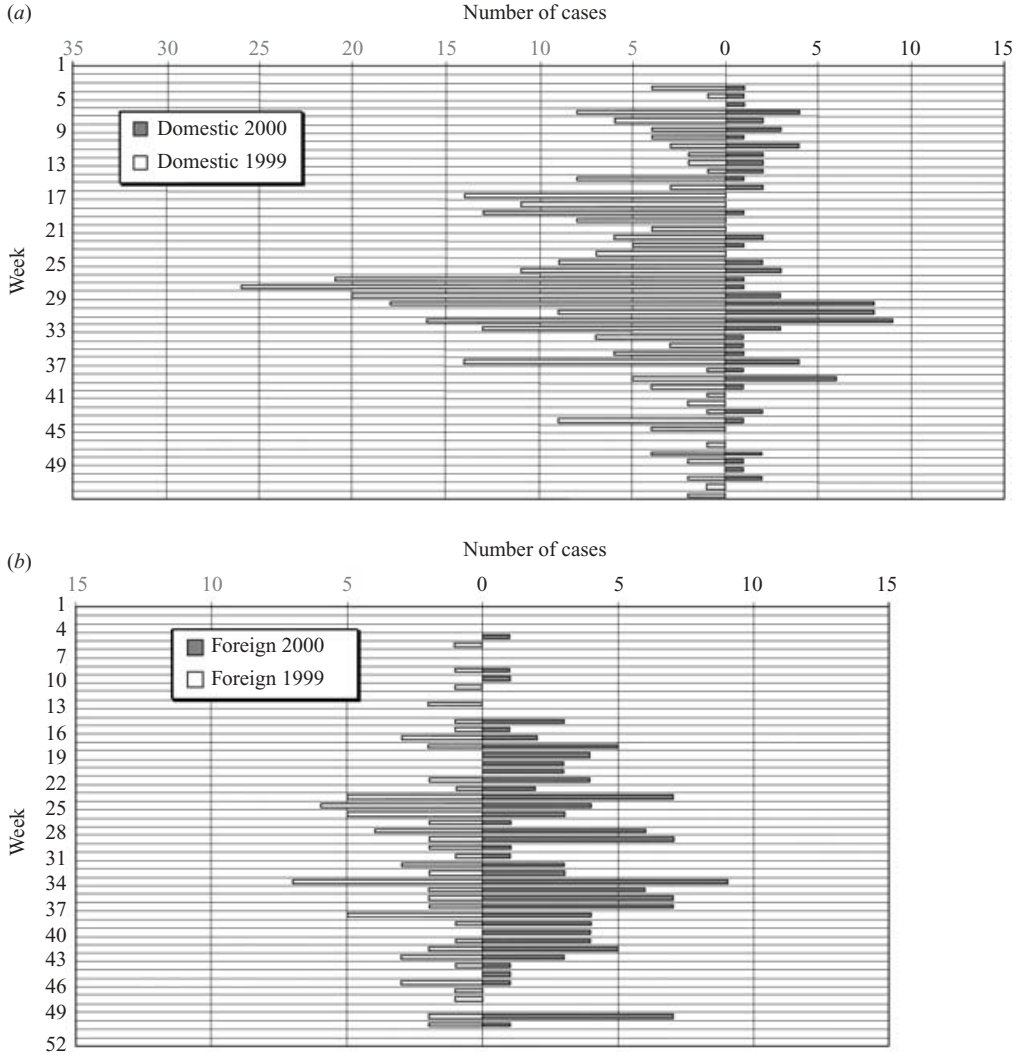


**Fig. 2.** Human infections of *Campylobacter* spp. in Iceland acquired from (*a*) domestic *vs.* (*b*) foreign exposure, by week, during the 1999 and 2000. Data based on diarrhoeatic specimens submitted to national surveillance programme administered by Landspitali University Hospital, Reykjavik, Iceland.

summertime peak in 2000 (weeks 29–31) but, its intensity and duration were greatly diminished relative to 1999 levels. Only week 31 had as many as 9 domestically acquired cases during 2000 as compared with 14 weeks in 1999 having 9 or more. Apart from the comparatively small peak in 2000, 10 of the weeks had 2 domestic cases, 17 weeks had only 1 domestic case and 14 weeks of the year had no domestic cases throughout Iceland. By way of comparison, the year 1999 had only 19 weeks with fewer than 4 domestically acquired cases per week. The largest numbers of domestically acquired infection in 1999 occurred at week 27, when 26 cases were reported.

The ratio of campylobacteriosis acquired during foreign travel (Fig. 2*b*) in proportion to the domestically acquired illness shifted from a comparatively small fraction to represent the majority of the human cases in Iceland (also shown in Fig. 1). In 1999, the ratio was 4·0 domestic cases to one foreign case (326/82). In 2000, that ratio was reduced to 0·7 domestic to 1·0 foreign case (92/130).

### Poultry sampling during 2000

During 2000, we began to focus on characterizing the roles of various sources and factors involved in transmitting *Campylobacter* spp. within the integrated poultry operations. Table 3 contains data on the detection and enumeration of the organism within poultry production operations. The organism was isolated from 82% of the faecal droppings taken from the grandparent breeder stock (148/180) which served to populate the commercial Iceland parent broiler breeder stock. The levels of campylobacter averaged about 300 cells (2·47 log 10) per g of faeces among the grandparent breeder birds. The breeder flocks were variably colonized, with eight groups of breeders being colonized in ~72% (417 of 579) of the droppings sampled and in none of 310 breeder droppings that were sampled from subsequent groups of breeder stock over the period 14 June to 24 July 2000. Among the 136 broiler caecal samples taken from 22 May to 6 June 2000, we found no colonization. From 30 June to 7 July 2000, we found 16 of 120 broiler caeca colonized by *Campylobacter* spp.

The presence of *Campylobacter* spp. on chicken transport crates was monitored (Table 3). Of the samples analysed, 10% (28/280) of the crate swabs yielded the organism. Phylogenetic relations must be determined to indicate if unique isolates were already on the crate surfaces when the birds were cooped for transport or whether the excretions of the transported birds were the source of crate contamination (K. L. Hiett, N. J. Stern; unpublished results).

### Poultry samples providing a measure of human exposure during 1999 and 2000

Table 4 contains a combination of qualitative and quantitative data pertinent to human exposure through poultry. During 9 August–26 October 1999, carcass rinse data indicated that approximately 62% of the broilers (1309/2099) were contaminated as assessed by qualitative methods described. From the beginning of June to the end of July 2000, we enumerated the levels of the organism in samples that represented human exposure. We observed levels of 4·97 log 10 cells of *Campylobacter* spp. per contaminated carcass on 30 June 2000 which may also have served to provide public exposure manifested during weeks 9 and 16 July 2000 (weeks 28 and 29), potentially resulting in increased human disease as shown in Figure 2. Likewise, we recorded contamination levels of 4·08 log 10 cells of *Campylobacter* spp. per carcass on 6 July 2000 and 17–28 July 2000 (weeks 28–30), selected processed flocks yielding greater than 3·3 log 10 cells of *Campylobacter* spp. per carcass. This increased public exposure on poultry provides a strong association related to increased human cases observed during that same period of time (Fig. 2). The highest frequency (18 of 18 weep samples) and highest levels (2·95 log 10 c.f.u./ml) of weep fluids found on 27 July 2000 may have contributed to the large number of weekly cases occurring during week 31 (Fig. 2).

## DISCUSSION

Among the farms which were processed in Abattoir A, there were inconsistent frequencies of *Campylobacter* spp. contamination associated with the number of flocks produced on those farms (Table 1). The smaller operations (Abattoir A, farms 3–5 and Abattoir B, 4 small farms), likewise, produced carcasses with a wide range in contamination frequency. Therefore, it appears that having multiple flocks or, only a single flock on a farm did not serve as a predictor for contamination. The carcass rinses from all 1999 flocks were analysed for *Campylobacter* spp. in Athens, Georgia and, detection was subject to variation in length of delivery time and sample transport temperatures. These factors are known to affect the viability of

Table 3. *Presence and levels of* Campylobacter *spp. in poultry production samples from grandparents, breeders, broiler chickens and crate samples, 22 May–28 July 2000*

| Date flock sampled (2000) | Sample type | No. of flocks | +/no. sampled | Average log 10 levels |
|---|---|---|---|---|
| 10 Jul. | Grandparent, faeces | 1 | 63/90 | 2·1/g |
| 10 Jul. | Grandparent, faeces | 1 | 85/90 | 2·8/g |
| 12 May | Breeder flock, faeces | 1 | 47/50 | 3·97/g |
| 15 May | Breeder flock, faeces | 1 | 17/51 | 0·64/g |
| 16 May | Breeder flock, faeces | 1 | 82/88 | 3·11/g |
| 22 May | Breeder flock, faeces | 1 | 66/90 | 1·90/g |
| 24 May | Breeder flock, faeces | 1 | 66/90 | 2·12/g |
| 30 May | Breeder flock, faeces | 1 | 46/90 | 0·94/g |
| 30 May | Breeder flock, faeces | 1 | 68/90 | 1·94/g |
| 30 May | Breeder flock, faeces | 1 | 25/30 | 1·92/g |
| 14 Jun. | Breeder flock, faeces | 1 | 0/80 | ND* |
| 14 Jun. | Breeder flock, faeces | 1 | 0/80 | ND |
| 21 Jun. | Breeder flock, faeces | 1 | 0/50 | ND |
| 4 Jul. | Breeder flock, faeces | 1 | 0/50 | ND |
| 24 Jul. | Breeder flock, faeces | 1 | 0/50 | ND |
| 22 May–6 Jun. | Broiler caeca | 34 | 0/136 | ND |
| 30 Jun. | Broiler caeca | 2 | 4/8 | 5·30/g |
| 3–5 Jul. | Broiler caeca | 5 | 0/20 | ND |
| 6 Jul. | Broiler caeca | 3 | 4/12 | 4·57/g |
| 7–14 Jul. | Broiler caeca | 6 | 0/24 | ND |
| 17 Jul. | Broiler caeca | 3 | 4/12 | 4·2/g |
| 19–24 Jul. | Broiler caeca | 6 | 0/24 | ND |
| 27 Jul. | Broiler caeca | 3 | 4/12 | 5·02/g |
| 28 Jul. | Broiler caeca | 2 | 0/8 | ND |
| 20–27 Jun. | Crate swabs | 5 | 0/50 | ND |
| 30 Jun. | Crate swabs | 2 | 10/20 | 3·92/ml |
| 3–5 Jul. | Crate swabs | 5 | 0/50 | ND |
| 6 Jul. | Crate swabs | 2 | 9/20 | 3·27/ml |
| 11–13 Jul. | Crate swabs | 4 | 0/40 | ND |
| 17 Jul. | Crate swabs | 2 | 6/20 | 0·83/ml |
| 19–24 Jul. | Crate swabs | 4 | 0/40 | ND |
| 27 Jul. | Crate swabs | 2 | 7/20 | 1·87 |
| 28 Jul. | Crate swabs | 2 | 0/20 | ND |

* ND, not detected at levels of less than log 1·5 (30) c.f.u. per g or per swab for all samples.

*Campylobacter* spp. and, the true frequency of contaminated carcasses was likely to have been even higher than we report (Table 2).

The data presented in Table 2 extend the unanswered question of how can flocks within a farm have inconsistent colonization? These flocks were tended by the same animal care attendants, provided equivalent husbandry practices to each flock, and were exposed to similar environmental *Campylobacter* spp. challenge. Other researchers [17, 18] have noted that spread from environmental sources might be sporadic. Broiler houses on these two farms sometimes were built to different construction standards and, the length of time between flocks might vary from only days, to more than 2 weeks. Clearly, more information is required to assess the contribution of these factors.

During the observed epidemic increases in frequency of campylobacteriosis in Iceland (Fig. 1), the public health authorities, the news media, the consumers and the poultry producers were seeking enhanced control measures. Iceland is a nation with a high level of literacy (>98%) and public involvement and, therefore, the issue became extensively disseminated in a short time. The word 'Campylobacter' became a household term and, the public health authorities were quickly

Table 4. *Frequency and levels of* Campylobacter *spp. contamination associated with processed poultry serving to provide potential human exposure in fresh chilled carcasses*

| Date | +/no. sampled | No. of lots | Average log 10 levels |
|---|---|---|---|
| Carcass rinses | | | |
| 9 Aug.–26 Oct. 1999 | 1309/2099 | 36 | 48 h transport, no enumeration |
| 8–28 Jun. 2000 | 0/130 | 13 | ND* |
| 30 Jun. 2000 | 10/20 | 2 | 2·97/ml or 4·97/carcass |
| 3–5 Jul. 2000 | 0/40 | 4 | ND |
| 6 Jul. 2000 | 5/20 | 2 | 2·08/ml or 4·08/carcass |
| 11–13 Jul. 2000 | 0/40 | 4 | ND |
| 17 Jul. 2000 | 2/20 | 2 | 1·60/ml or 3·60/carcass |
| 19 Jul. 2000 | 0/20 | 2 | ND |
| 20 Jul. 2000 | 1/10 | 1 | 1·50/ml or 3·50/carcass |
| 24 Jul. 2000 | 2/10 | 1 | 1·35/ml or 3·3/carcass |
| 26 Jul. 2000 | 10/10 | 1 | 2·07/ml or 4·07/carcass |
| 27 Jul. 2000 | 10/20 | 2 | 2·70/ml or 4·7/carcass |
| 28 Jul. 2000 | 1/20 | 2 | 2·33/ml or 4·33/carcass |
| Carcass weeps | | | |
| 6 Jul. 2000 | 13/36 | 2 | 0·71/ml |
| 6 Jul. 2000 | 4/18 | 1 | 0·28/ml |
| 17 Jul. 2000 | 1/18 | 1 | 0·06/ml |
| 17 Jul. 2000 | 11/18 | 1 | 0·67/ml |
| 20 Jul. 2000 | 0/18 | 1 | ND |
| 24 Jul. 2000 | 17/18 | 1 | 1·57/ml |
| 26 Jul. 2000 | 17/18 | 1 | 1·81/ml |
| 27 Jul. 2000 | 18/18 | 1 | 2·95/ml |

* ND, not detected at levels of less than log 1·5 (30) c.f.u. of all samples.

held accountable. There is additional evidence from Hiett et al. [22] who describe the phylogenetic relationships of the human, poultry and non-poultry veterinary isolates as analysed by DNA sequencing technology. This evidence confirms the prominent role of poultry in transmission of human disease.

A fundamental change in food consumption patterns and practices occurred simultaneously with the onset of the epidemic. During this time period, the public demand for poultry products increased. This was coupled with market driven desires for chilled (refrigerated fresh) product, as opposed to purchase of frozen poultry products. Prior to 1996 only frozen products had been marketed in Iceland. After 1996, markets increasingly demanded fresh poultry, which contained comparatively higher levels of campylobacter per carcass and provided greater public exposure to the contaminated products [13]. During production, flocks which were detected (via cloacal swabs) as *Campylobacter* spp. colonized at 4 weeks of age were processed at the end of the week (on Fridays) to minimize cross-contamination to flocks presumed not previously colonized. Carcasses from these birds were then frozen and marketed. Freezing of carcasses causes a 10–100 fold reduction in levels of *Campylobacter* spp. cells, compared with carcasses that are maintained under refrigeration [13].

Additionally relevant, appropriate hygienic poultry handling and preparation of poultry may not have been traditionally taught to food handlers in Iceland. Poultry is currently occupying a more prominent portion of the Iceland diet, although only at a fraction ($\sim$33%) of the US per capita consumption. Iceland has effectively reduced salmonella contamination on poultry products to non-detectable levels in both their poultry production and their processed products (unpublished data). Consequently, consumer awareness of poultry-borne pathogens may previously have been lacking and, in 2000, a dramatic flurry of public education was provided. Additionally, the poultry farmers began to improve biological security measures in an attempt to control spread of the organism from the environment to the flocks. The Iceland poultry industry by official ruling began to freeze process lots

of broiler carcasses which came from flocks testing positive for *Campylobacter* spp. Flocks testing positive 1 week prior to slaughter and from the subsequent two flocks raised in the same broiler house, were frozen after processing. The substantially lower prices paid to producers for frozen lots has been an important factor reinforcing the emphasis now placed on broiler house clean-out, disinfection and the practice of biological security measures.

Cloacal swabs, as used in the present study, are fundamentally an estimate to detect campylobacter colonization in broiler poultry flocks [19]. Some of the flocks may have been colonized at 4 weeks of age, but were not detected. Some flocks may have become colonized after sampling at 4 weeks and arrived at the processing plant (at approximately 5·5 weeks of age) with high levels of contamination [20]. A significant reduction in cases of domestically acquired campylobacteriosis was observed in 2000. As illustrated in Figure 2, it is quite likely that the combined efforts of the public health authorities and the willingness of the poultry industry to implement improved broiler house hygiene, to tighten biological security and to freeze products when flocks were detected positive, appear to have controlled the epidemic.

The majority of the weeks which were monitored during 2000 indicated that a 'background infection frequency' of 0, 1, or only 2 cases occurred weekly. This observation supports the contention that additional sources are likely to be involved in human infection. Poultry is not the only source of *Campylobacter* spp. for the human population. It does appear reasonable that poultry serves to amplify human exposure to the organism, but with lower broiler flock prevalence and reduced levels of public exposure for a variety of reasons, the relative role of other sources becomes more significant.

Egg-borne transmission of *Campylobacter* spp. has been reported as one route of transmission to broiler chickens [21]. In that report, identical *flaA* SVR DNA sequences and ribotype patterns from isolates were characterized from totally independent breeder and their progeny broiler flocks located $>20$ miles apart. This consistent observation which was made for two separate breeder/broiler operations in separate states within the United States, strongly suggests such transmission to broilers can occur. Arguing against such egg-borne transmission are the data from this current study, indicating that the breeders were highly colonized while less than 5% (12/254) of the offspring broiler caeca were positive (Table 3). Low broiler flock prevalence occurred during the sampling period of May to the end of July 2000. This may also suggest that different hatchery practices and conditions may effect transmission, that not all *Campylobacter* spp. isolates may be capable of inducing egg-borne transmission, that a certain level of intestinal colonization is required, or that breeder bird age may influence egg-borne transmission. A more in-depth study is underway to address these possibilities.

External contamination of the broilers increased from an average of 1200 cells of *Campylobacter* spp. per carcass on the production farm to over 12 million cells per carcass after bird transport [20]. The source of such an increase in contamination is likely to be the birds defecating onto one another, as well as upon the crates. Hygienic measures have been enacted in Iceland to reduce or eliminate the presence of the organism on crates, thus, controlling the potential for cross-contamination.

The circumstances in Iceland poultry operations changed dramatically during 2000. These changes coincided with reductions in human disease. Both weep fluid samples and carcass rinse samples provide a measure of consumer exposure. Given the considerable number of significant interventions adopted in the Iceland poultry industry in the period 1999–2000, it is not possible to discern which, if any, of these interventions played an important role in reducing the human case burden during this period. It is reasonable to assume, *a priori*, that interventions which reduced flock prevalence (through improved on-farm biological security) and which reduced the pathogen load on carcasses (through emphasis on cleaning and disinfection of broiler houses between flocks, effective cleaning and disinfection of live haul crates to reduce cross-contamination and freezing of carcasses on some positive lots) contributed to reduced consumer exposure. Since both types of interventions co-incided in 1999–2000, there is no immediate basis for assigning credit to either type of intervention. Clearly there may be other interventions (e.g. changes in consumption and consumer handling practices) or natural phenomena (e.g. changes in the environmental sources of campylobacter in the period 1999–2000) which could also explain the dramatic decrease in the human health burden. This study gives additional insight into the nature of the exposure and provides important baseline information for further characterization of this closed poultry production and consumption system. Although we cannot yet quantitatively assign importance to any of the many changes which are

known or are presumed to have occurred in 1999–2000, we continue to quantify those factors which contribute to transmission of the organism in this system.

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