Conference for Food Protection
2018 Issue Form

Issue: 2018 III-031

Council Recommendation:	Accepted as Submitted	_____	Accepted as Amended	_____	No Action	_____
Delegate Action:	Accepted	_____	Rejected	_____		

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Issue History:

This is a brand new Issue.

Title:

Amend Food Code – Date Marking Requirements on Consumer Deli Meat

Issue you would like the Conference to consider:

A recommendation is being made to amend the Food Code to require food establishments to disclose original date marking on deli meats cut and packaged within the food establishment.

Public Health Significance:

Listeria monocytogenes continues to be an organism of public health significance, with the Center for Disease Control and Prevention website providing information estimating an average of 1,600 illnesses and 260 deaths per year. Food such as deli meats, which have no further lethality step and are stored at refrigerated temperatures carry significant risk. Regulatory policy has been shaped over the last 10 years, including testing and cleaning requirements to reduce the risk[1].

Consumer food safety practices have been previously studied using risk assessment and survey data. Consumer refrigeration storage temperatures have been found to be an average of 42.8°F, with a range between 30.5°F and 52.5°F[2]. Growth of listeria, combined with cross contamination of product in home kitchens, can lead to increased disease burden if not managed[3].

Current regulatory requirements work to reduce risk of listeria monocytogenes by requiring ready to eat foods to be consumed or discarded within 7 days, with date the packaged opened being counted as day 1 (3-501.17). This means that deli meats which were sliced in an establishment would have to be used or discarded within 7 days of package being opened if the meat remained in the food establishment. However, there is no requirement for that date to be disclosed to a consumer when purchasing fresh sliced deli meats.

Consumer messaging currently recommends deli meats being used or discarded within 3-4 days of purchase. With the current regulatory requirements, deli meats could be opened for six days and then sold directly to a consumer who does not have knowledge of the original

opening date. Providing original date marking to the consumers allows for consumers to make a more informed risk management decision.

References (Noted above with superscript numerals)

1. Gottlieb et al, Clinical Infectious Diseases, Volume 42, Issue 1, 1 January 2006, Pages 29-36
2. James et al, International Journal of Refrigeration; Volume 15, Issue 5, 1992, Pages 299-306
3. Lianou et al, Food Microbiology 24 (2007) 433-443

Recommended Solution: The Conference recommends...:

That a letter be sent to the FDA requesting the most current edition of Food Code, Section 3-501.17 be amended to include the requirements for consumer portions of sliced deli meats to include the original date the package was opened.

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Supporting Attachments:

- "Multistate Outbreak of Listeriosis Linked to Turkey Deli Meat"
- "Consumer handling of chilled foods: Temperature performance"
- "Behavior of Listeria monocytogenes at 7 C in commercial turkey breast"

MAJOR ARTICLE

Multistate Outbreak of Listeriosis Linked to Turkey Deli Meat and Subsequent Changes in US Regulatory Policy

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Background. Listeriosis, a life-threatening foodborne illness caused by *Listeria monocytogenes*, affects ~2500 Americans annually. Between July and October 2002, an uncommon strain of *L. monocytogenes* caused an outbreak of listeriosis in 9 states.

Methods. We conducted case finding, a case-control study, and traceback and microbiological investigations to determine the extent and source of the outbreak and to propose control measures. Case patients were infected with the outbreak strain of *L. monocytogenes* between July and November 2002 in 9 states, and control patients were infected with different *L. monocytogenes* strains. Outcome measures included food exposure associated with outbreak strain infection and source of the implicated food.

Results. Fifty-four case patients were identified; 8 died, and 3 pregnant women had fetal deaths. The case-control study included 38 case patients and 53 control patients. Case patients consumed turkey deli meat much more frequently than did control patients ($P = .008$, by Wilcoxon rank-sum test). In the 4 weeks before illness, 55% of case patients had eaten deli turkey breast more than 1–2 times, compared with 28% of control patients (odds ratio, 4.5; 95% confidence interval, 1.3–17.1). Investigation of turkey deli meat eaten by case patients led to several turkey processing plants. The outbreak strain was found in the environment of 1 processing plant and in turkey products from a second. Together, the processing plants recalled >30 million pounds of products. Following the outbreak, the US Department of Agriculture's Food Safety and Inspection Service issued new regulations outlining a *L. monocytogenes* control and testing program for ready-to-eat meat and poultry processing plants.

Conclusions. Turkey deli meat was the source of a large multistate outbreak of listeriosis. Investigation of this outbreak helped guide policy changes designed to prevent future *L. monocytogenes* contamination of ready-to-eat meat and poultry products.

Listeriosis, a life-threatening, primarily foodborne illness caused by *Listeria monocytogenes,* affects an estimated 2500 people in the United States annually [1]. Immunocompromised persons, elderly persons, and pregnant women and their fetuses or newborns are at highest risk for infection [2]. Listeriosis most commonly presents as an invasive illness with bacteremia or meningitis and has a case-fatality rate of 20% [3]. In pregnancy, listeriosis may lead to fetal death, preterm labor, or invasive listeriosis in the newborn child [2]. We describe the investigation of a large, multistate listeriosis outbreak in 2002 that was linked to turkey deli meat and prompted new changes in US food regulatory policy.

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METHODS

Case finding. During July and August 2002, there were 22 cases of listeriosis reported in Pennsylvania, a nearly 3-fold increase over baseline. Clinical isolates were sent to the Centers for Disease Control and Prevention (CDC; Atlanta, GA) for molecular subtyping by PFGE, which identified a cluster of cases caused by a single *L. monocytogenes* strain. The CDC asked health departments in the northeast United States to conduct active case finding, prompt reporting of listeriosis cases, and retrieval of clinical isolates for rapid PFGE testing via PulseNet. PulseNet, the National Molecular Subtyping Network for Foodborne Disease Surveillance, is the CDC-coordinated network of public health laboratories that perform PFGE on foodborne bacteria [4]. The network permits rapid electronic comparison of PFGE patterns through a database at the CDC. PulseNet staff compared the electronically posted PFGE patterns from all public health laboratories to the PFGE pattern from the Pennsylvania cluster.

Definitions. Listeriosis was defined as illness in a person from whom *L. monocytogenes* was isolated (from any clinical specimen). The 2-enzyme PFGE pattern from the Pennsylvania listeriosis cluster was designated as the outbreak pattern. Infection with the outbreak strain was defined as having listeriosis with an *L. monocytogenes* isolate yielding a PFGE pattern indistinguishable from the outbreak pattern. For the purposes of case counting, a mother-neonate pair was considered to be 2 cases if both mother and newborn were ill and had culture-confirmed *L. monocytogenes* infection; if only one of the pair was ill with culture-confirmed *L. monocytogenes* infection, only 1 case was counted. However, for the purposes of the case-control study, a mother-neonate pair was always considered to be a single case. For an ill pregnant woman with an associated fetal death (stillbirth or miscarriage), only the mother's illness was counted as a case.

Case-control study. We conducted a case-control study to identify the contaminated food source. A case patient was defined as a person with culture-confirmed listeriosis between 1 July and 30 November 2002, whose infection was caused by the outbreak strain. A control patient was defined as a person with culture-confirmed listeriosis between 1 July and 30 November 2002, whose infection was caused by any other non-outbreak strain of *L. monocytogenes,* and who was from a state with at least 1 case patient. Case patients and control patients were interviewed with a standard questionnaire that addressed medical and food histories during the 4 weeks preceding culture for *L. monocytogenes* (all patients except mother-neonate pairs) or delivery date (mother-neonate pairs). The questionnaire included >70 specific food items.

Statistical methods. Statistical analyses were performed with SAS software, version 8.2 (SAS). Food exposures of case patients and control patients were compared using the Wilcoxon rank-sum test. ORs and exact 95% CIs were calculated, controlling for state or city by conditional logistic regression.

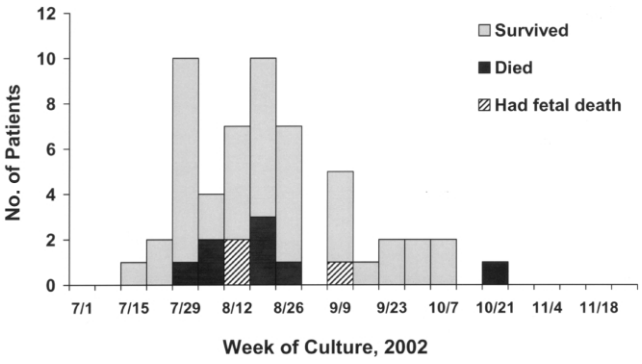

Figure 1. Number of patients infected with the outbreak strain of *Listeria monocytogenes,* by week of culture, July to November 2002 ($n = 54$).

Traceback investigation. Health and regulatory officials traced back the food implicated in the case-control study. A purchase site was defined as any delicatessen, grocery store, or restaurant where the implicated food consumed by a case patient was purchased. Inventories and invoice information were obtained from purchase sites to determine which processing plants had supplied the food.

Plant investigations. Plants A, B, C, and D were investigated by teams led by the US Department of Agriculture's Food Safety and Inspection Service (FSIS). Noncompliance reports, production and construction records, and records of product and environmental sampling for *Listeria* species maintained by plant staff were reviewed. Environmental samples were collected aseptically by cellulose sponge. Unopened food products were collected from each plant's "shelf-life" stock and from current production. Recalled products from plant A were also collected.

Laboratory investigation. PFGE testing was performed in accordance with the standard PulseNet protocol using restriction endonucleases *Asc*I and *Apa*I [5]. Study isolates were confirmed at the CDC to be *L. monocytogenes* by AccuProbe (GenProbe), ribotyped using the RiboPrinter microbial characterization system (Qualicon), and serotyped using standard methods [6].

Food samples were transported at 4°C and cultured for *L. monocytogenes* using standard methods [7]. Environmental sponges were transported at 4°C in Dey-Engley neutralizing media and tested using the standard methods mentioned above. *L. monocytogenes* isolates were subtyped by PFGE in the same manner as were human isolates. PFGE patterns were electronically compared with human isolate patterns through PulseNet.

RESULTS

Case finding and description. Between 1 July and 30 November 2002, there were 188 patients with *L. monocytogenes*

infection identified in 9 states: Pennsylvania, New York, New Jersey, Delaware, Maryland, Connecticut, Massachusetts, Michigan, and Illinois. Of these patients, 54 were infected with the outbreak strain, 1 was infected with a strain that resembled the outbreak strain but differed by 1 band on the *Apa*I PFGE analysis, and 122 were infected with unrelated *L. monocytogenes* strains; no isolates were available for 11 patients. The dates of specimen collection for the 54 case patients ranged from 18 July to 26 October (figure 1). Of the ~2000 human *L. monocytogenes* isolates in the PulseNet database from the 6 years before the outbreak, only 20 were indistinguishable from the outbreak PFGE pattern (PulseNet pattern designation GX6A16.0235-GX6A12.0003). All outbreak strain isolates tested were serotype 4b and ribotype DUP-1044.

Characteristics of the 54 case patients and 122 control patients are shown in table 1. Eight case patients (15%) were pregnant women, and 4 (7%) were neonates. Prior medical information was available for 41 of the 42 non–maternal-neonatal case patients. Thirty patients (71%) had the following medical conditions or factors, which are considered to be immunocompromising or predisposing for listeriosis: hematologic malignancy (5 patients), corticosteroid use (4 patients), HIV infection and/or AIDS (4 patients), solid malignancy (3 patients), diabetes (3 patients), liver disease (3 patients), inflammatory bowel disease (3 patients), organ transplantation (2 patients), end-stage renal disease (1 patient), rickets and malnutrition (1 patient), and sickle-cell anemia (1 patient). Most elderly case patients also had an underlying immunocompromising condition; only 4 (7%) of the 54 case patients were aged ⩾65 years and were not immunocompromised. Seven patients (13%) were aged 1–64 years and were not pregnant or immunocompromised. Case patients were more likely than control patients to be pregnant or young and healthy (i.e., aged 1–64 years without predisposing medical conditions [$P < .001$]).

Eight (15%) of 54 case patients died. Of these, 4 patients were aged 1–64 years and were immunocompromised, 3 were aged ⩾65 years and were also immunocompromised, and 1 was a neonate. The case-fatality rate among case patients was lower than that among control patients (15% vs. 26%), although not significantly ($P = .14$). However, among immu-

Table 1. Characteristics of case patients and control patients, 1 July–30 November 2002.

Characteristic	Case patients ($n = 54$)	Control patients ($n = 122$)
State and/or city		
Pennsylvania	15 (28)	27 (22)
New York (excluding New York City)	9 (17)	20 (16)
New York City	12 (22)	12 (10)
New Jersey	5 (9)	19 (16)
Delaware	4 (7)	1 (1)
Maryland	2 (4)	10 (8)
Connecticut	1 (2)	8 (7)
Michigan	1 (2)	10 (8)
Massachusetts	3 (5)	12 (10)
Illinois	2 (4)	3 (2)
Sex[a]		
Male	32 (59)	54 (45)
Female	22 (41)	67 (55)
Pregnant	8 (15)	4 (3)
Neonate	4 (7)	9 (7)
Immunocompromised, by age[b]		
⩾65 years	13 (25)	45 (45)
1–64 years	17 (32)	30 (30)
Not immunocompromised, by age[b]		
⩾65 years	4 (7)	7 (7)
1–64 years	7 (13)	4 (4)
Death	8 (15)	32 (26)
Pregnancy complication(s)	6	3
Fetal death	3	2

NOTE. Data are no. (%) of patients.

[a] Sex was unknown for 1 control patient.

[b] Prior medical information was unavailable for 1 case patient and 23 control patients.

Table 2. Selected food exposures among case patients and control patients.

Food item, by frequency of consumption during 4 weeks before illness	No. (%) of case patients[a]	No. (%) of control patients[a]	P[b]	OR[c]	95% CI	P
Turkey breast deli meat from restaurant or deli counter			.008			
More than 1–2 times	21 (55)	14 (29)		4.5	1.3–17.1	.012
1–2 times	8 (21)	14 (29)		1.1	0.3–4.7	1.000
Never	9 (24)	21 (43)		1.0	Referent	…
Other turkey deli meat[d] from restaurant or deli counter			.030			
More than 1–2 times	6 (17)	3 (6)		3.0	0.6–20.3	.240
1–2 times	3 (8)	1 (2)		5.7	0.4–305.3	.260
Never	27 (75)	45 (92)		1.0	Referent	…
Loose lettuce			.030			
More than 1–2 times	8 (22)	22 (44)		0.3	0.1–0.9	.039
1– 2 times	10 (27)	10 (20)		0.7	0.2–2.4	.706
Never	19 (51)	18 (36)		1.0	Referent	…

[a] In some cases, data were not available for all patients.
[b] By Wilcoxon rank-sum test. The Wilcoxon P value reflects comparisons of all levels of food consumption.
[c] Adjusted odds ratio, controlling for state or city (for New York City), for categorized levels of food consumption.
[d] For example, turkey ham or turkey pastrami.

nocompromised and neonatal patients, the case-fatality rates were similar: 24% for case patients versus 29% for control patients. Of the 8 pregnant case patients, 6 (75%) had complications: 3 had fetal deaths, 2 gave birth to infants with listeriosis (1 birth was premature), and 1 woman gave birth prematurely to an infant without listeriosis. Complications among the 4 pregnant control patients were similar, with 2 fetal deaths and 1 birth of an infant with listeriosis.

Institution-associated infections. Some listeriosis infections appeared to have been acquired nosocomially, presumably from food served at medical institutions. Although we did not explicitly ask about prior hospitalization, review of written comments on 122 interview forms revealed that 22 patients (18%) reported staying in a hospital, nursing home, or rehabilitation center in the 4 weeks before submitting specimens for *L. monocytogenes* culture. Dates of institutionalization were recorded for 15 patients, most of whom had been in the institution for almost the entire 4-week period (median, 28 days [range, 5–28 days]). We obtained institution menus or interviewed dietary staff for 10 patients. Turkey deli meat (not specified as being "heated" or "hot") was offered to 8 of these patients during their stays in the institutions. An additional 3 patients, for whom hospital menus were not available, reported being served turkey deli meat while hospitalized.

Case-control study. Questionnaire responses were obtained from 91 (91%) of 100 eligible case patients and control patients. We excluded patients identified after the announcement of case-control study findings on 4 October 2002 ($n = 73$). Three mother-neonate pairs were counted as single patients. The final epidemiologic analysis included data obtained from 38 case patients and 53 control patients (table 2). Infection with the outbreak strain was strongly associated with consumption of precooked turkey breast products sliced at the deli counter of groceries and restaurants ($P = .008$, by Wilcoxon rank-sum test). The Wilcoxon rank-sum test captures differences in the frequency of consumption. Overall, 55% of case patients ate turkey deli meat more than 1–2 times in the 4-week period, compared with 29% of control patients (OR, 4.5; 95% CI, 1.3–17.1). Other turkey deli meats, such as turkey ham and turkey pastrami, were eaten by fewer patients and primarily by persons who also ate turkey breast products. Therefore, it was difficult to exclude risk from these deli meats as well. No other single food item was significantly associated with outbreak strain infection, except for lettuce, which was protective.

Traceback. Interviews of the first 29 case patients who had eaten turkey deli meat identified 80 purchase sites (mean, 3 sites per person [range, 1–8 sites]). None of the patients recalled the brand names of all turkey deli meat purchased. Sixty-eight of the 80 purchase sites could be located; 57 (84%) of these

Table 3. Results of testing of the environment and previously unopened turkey products at 4 turkey processing plants.

	No. of environmental samples			No. of unopened product samples		
Plant	Total tested	LMP	With outbreak strain	Total tested	LMP	With outbreak strain
A	57	25	2	108	2[a]	0
B	48	1	0	18	2	2
C	50	0	…	6	0	…
D	51	0	…	6	0	…

NOTE. LMP, *Listeria monocytogenes*–positive.
[a] Two different strains were identified; both were also found in the plant's environment and isolated from 2 control patients during the outbreak.

Table 4. Previously opened turkey product testing yielding the outbreak strain of *Listeria monocytogenes*.

Product	How and where product was obtained	Brand on product label	Plant(s) supplying turkey to purchase site
1	Routine inspection of New York City delicatessen	Unknown	Plants A and B
2	Traceback evaluation of New York City delicatessen	Plant C	Plants A, B, C, and D
3	Traceback evaluation of New York City delicatessen	Plant B	Plant B
4	Case patient's sandwich, Massachusetts	Plant A[a]	Plants A and B

[a] A relative of the patient called the market where the turkey was purchased and, after describing its label information, was told the turkey was a plant A product.

were investigated, with identification of products from >50 turkey processing plants. FSIS evaluated the 15 most frequently identified plants, using a standardized survey to address such factors as previous food safety infractions, history of recent construction, and records of in-plant *Listeria* species sampling. On the basis of these survey results, plants A, B, C, and D were found to warrant in-plant investigation.

Processing plant and marketplace investigations. Multiple *L. monocytogenes* strains were identified in the environment of plant A (table 3). Two samples, both collected from floor drains, yielded the outbreak strain. Another isolate yielded a PFGE pattern differing from the outbreak pattern by 1 band, which was indistinguishable from that of 1 patient's isolate during the outbreak. Records of the plant's in-house environmental sampling in the room where cooked turkey was handled revealed an increase in the number of samples that yielded *Listeria* species during July and August 2002, compared with previous months and with the same months in 2001. This increase in positive test results coincided with a large construction project performed in the same room. Testing of previously unopened turkey products from plant A did not yield the outbreak strain (table 3). However, it did yield 2 *L. monocytogenes* strains that were also found in the plant's environment and that were isolated from 2 control patients. The outbreak strain of *L. monocytogenes* was not recovered from any environmental samples from plant B. However, the outbreak strain was isolated from 2 of 18 previously unopened turkey samples produced by plant B. Plant B is located ~30 miles from plant A.

Sampling of previously opened packages of turkey yielded the outbreak strain on 4 occasions (table 4). At the time of interview, only 1 case patient still had a sample of the turkey eaten before illness. A sandwich made with the same turkey deli meat consumed on 2 previous days was left in a cooler when an otherwise healthy young man was admitted to a hospital with *L. monocytogenes* bacteremia. The sandwich was retrieved from the cooler 2 weeks later, and *L. monocytogenes* with the outbreak PFGE pattern was isolated from the turkey. Invoice records revealed that only turkey products from plant A and plant B were sold at the market where the patient purchased the turkey.

Regulatory outcomes. In response to these findings, plants A and B voluntarily suspended operations and began intensive cleanup, and together they recalled >13.5 million kg (>30 million lbs) of ready-to-eat poultry products. The first recall occurred on 9 October 2002. In December 2002, immediately following the outbreak, FSIS issued a policy directive outlining an intensified microbiological testing program for ready-to-eat meat and poultry plants [8]. Over the following year, FSIS completed a risk assessment for *L. monocytogenes* in deli meats [9] and finalized and issued a new regulation aimed at further reducing *L. monocytogenes* contamination of ready-to-eat meat and poultry products [10].

DISCUSSION

We investigated a multistate outbreak of listeriosis that affected 54 persons and caused 8 deaths and an additional 3 fetal deaths. An epidemiologic investigation linked outbreak strain infection with consumption of precooked, ready-to-eat turkey deli meat, making this the third multistate listeriosis outbreak related to ready-to-eat meat and poultry products in recent years [11, 12]. The outbreak strain of *L. monocytogenes* was found in the environment of plant A and in turkey breast products from plant B. Both plants suspended production and together recalled >13.5 million kg (>30 million lbs) of products, resulting in one of the largest meat recalls in US history. In the year following the outbreak, federal regulators issued new, more-stringent rules designed to prevent further *L. monocytogenes* contamination of ready-to-eat meat and poultry products [8, 10].

L. monocytogenes has 2 unique characteristics that influence its transmission to humans through ready-to-eat foods. First, it is a tenacious colonizer that favors moist, cool environments, such as food processing plants; eradication is difficult [13]. Second, although it is easily killed by cooking, *L. monocytogenes* multiplies readily at refrigeration temperatures, whereas most other competing microflora do not [14]. A small amount of

L. monocytogenes contamination at a processing plant that occurs after cooking but before packaging may lead to a large infectious dose being delivered to a susceptible consumer, because of multiplication of bacteria during storage [15]. Ready-to-eat poultry products, such as turkey deli meat, provide a particularly favorable environment for growth of *L. monocytogenes* during refrigeration [15]. Recent risk assessment models have estimated that, among ready-to-eat foods, deli meats and nonreheated hot dogs have the highest risk of listeriosis per serving [16].

Outbreak investigations can highlight high-risk food items and target regulatory, industry, and public health action. Our investigation led us to 2 plants. Plant B was likely linked to illnesses, because the outbreak strain was found in its turkey products. It is also possible that plant A was linked to illnesses, because the outbreak strain and several other *L. monocytogenes* strains were found throughout the main room where unpackaged turkey products were handled. In addition, 2 nonoutbreak strains were found in both the plant environment and in packaged turkey products, demonstrating the likelihood of ongoing contamination of products within the plant. The geographic proximity of plants A and B could allow introduction of a shared strain by receiving raw turkey products from the same slaughterhouse, purchasing equipment from the same source, or having employees in common [17]. A better understanding of the ecology of *L. monocytogenes* strains in ready-to-eat food plants would help guide efforts to prevent *L. monocytogenes* contamination of high-risk products.

Findings from this investigation helped guide FSIS in developing its new regulatory policy [8, 10]. Under these regulations, plants producing high-risk, ready-to-eat meat and poultry products must develop scientifically validated *L. monocytogenes*–control programs, which are stratified according to the number of control measures taken. One such measure is the institution of pathogen-elimination treatments after the product has been packaged, such as postpackaging heat treatments or "pasteurization" [18, 19]. Irradiation has been shown to be effective as a postpackaging treatment in studies, yet it has not been approved by the US Food and Drug Administration for use with ready-to-eat meat and poultry [20]. Additives that suppress the growth of *L. monocytogenes* in products are another strategy [21]. Plants with less rigorous control programs, particularly those relying solely on sanitation, are placed under an intensified microbiological testing program by FSIS. The new regulations also clarify, for the first time, that recalls can be based on identification of *L. monocytogenes* on equipment, not just in products, and that plant-generated microbiological testing data must be shared with FSIS officials.

A 2003–2004 FSIS survey revealed that the percentage of plants using new technologies to kill or suppress the growth of *L. monocytogenes* inside packaging has increased dramatically since release of the first FSIS policy changes, which were made in December 2002, as has the number of plants testing the processing environment for *L. monocytogenes* [22]. More than 87% of the >2900 establishments surveyed had made at least 1 change in their food safety process [22]. One year after the outbreak, FSIS reported a 25% decrease in the number of *L. monocytogenes*–positive samples detected by means of its regulatory testing program between January and September 2003, compared with 2002 [23]. Most importantly, preliminary 2004 national surveillance data revealed a 40% decrease in the incidence of human listeriosis, compared with 1996–1998, with 2.7 listeriosis cases per million persons [24]. This rate approaches the 2005 national objective of cutting listeriosis incidence to 2.5 cases per million persons [25].

In addition to regulatory and industry changes, another important aspect of listeriosis control is educating high-risk populations and their food preparers about high-risk foods, such as deli meats and hot dogs, soft cheeses made from unpasteurized milk, and smoked seafood [26]. Current recommendations to prevent foodborne listeriosis can be found at the CDC's Web site [27]. Prevention messages are straightforward for pregnant women and immunocompromised patients, who have a markedly increased risk of listeriosis [28]. Older age has long been considered a risk factor for listeriosis [2]; however, most patients with listeriosis aged ⩾65 years that we identified (93%) had other immunocompromising conditions, a finding in other outbreak investigations [29, 30]. Interestingly, patients infected with the outbreak strain were significantly more likely to be either young and healthy or pregnant than were patients with sporadic listeriosis. High doses of *L. monocytogenes* have been linked to illness in healthier people, yet primarily in the context of febrile gastroenteritis [31]. The role of organism pathogenicity in determining populations affected by invasive listeriosis is poorly understood [32, 33].

Although the precise risk to older persons may be difficult to define, institutions providing food to any at-risk population, including elderly persons, should implement appropriate policies regarding deli meats and other high-risk foods to reduce the risk of listeriosis. Several hospitalized or institutionalized patients identified during this outbreak probably acquired foodborne *L. monocytogenes* infection nosocomially, which is a scenario that has been documented previously [34, 35]. These findings indicate an urgent need for changes in these health care settings.

Finally, this investigation demonstrates the importance of PFGE subtyping through PulseNet in the detection and investigation of listeriosis outbreaks, as was recently discussed by Olsen et al. [12]. In 2003, the Council of State and Territorial Epidemiologists issued a position statement calling for prompt interviewing of all patients with listeriosis with a nationally standardized form and expedited referral of *L. monocytogenes*

isolates from clinical laboratories to state public health laboratories for real-time PFGE subtyping [36]. We encourage clinicians and laboratories to promptly report all cases of listeriosis to health departments, and we encourage states to adopt the Council of State and Territorial Epidemiologists' proposal.

In conclusion, our investigation linked a large outbreak of listeriosis to turkey deli meat and reinforced findings of previous outbreaks [11, 12] and risk models [9, 16] that ready-to-eat meat and poultry products continue to be a concern for at-risk populations. This investigation helped stimulate and guide new regulatory policies designed to prevent contamination at processing plants that produce these products. Industry surveys and national surveillance data already suggest a positive impact. Additional prevention measures implemented by institutions that serve high-risk populations could complement regulatory and industry efforts to reduce *L. monocytogenes* infections nationwide.

LISTERIOSIS OUTBREAK WORKING GROUP

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Consumer handling of chilled foods: Temperature performance

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Chilled foods are stored for periods of between a few hours and many days in domestic refrigerators. However, there are little published data on the temperature performance of domestic refrigerators within the home. A survey has been taken in 252 households in the UK and some of the results are presented in this paper. The refrigerators investigated in the survey were found to have an overall mean temperature of approximately 6°C, which ranged from 11.4 to −0.9°C. Temperature ranges over the whole refrigerator varied from 4.5 to 30.5°C with 3.7% of the total being warmer than 20°C. On average 29.9% of refrigerators operated below 5°C and 66.7% operated below 7°C. Few refrigerators (7.3%) ran, on average, above 9°C. No refrigerator characteristic (apart from type) could be related to temperatures or temperature distribution in the refrigerators investigated.
(Keywords: domestic refrigerators; temperature; chilled food)

Manipulation des produits réfrigérés par les consommateurs: Température assurée

Les aliments réfrigérés sont entreposés dans les réfrigérateurs domestiques, pour des périodes allant de quelques heures à plusieurs jours. Cependant, peu de données ont été publiées sur la performance des réfrigérateurs domestiques en matière de température, au domicile des consommateurs. Une enquête a été effectuée auprès de 252 ménages au Royaume Uni, et on présente quelques résultats dans cet article. Les réfrigérateurs considérés dans l'enquête avaient une température globale moyenne d'approximativement 6 deg C (elle variait de 11,4 à −0,9 deg C). Les plages de température globale allaient de 4,5 à 30,5 deg C, mais pour 3,7% des réfrigérateurs, la température était supérieure à 20 deg C. En moyenne, 29,9% des réfrigérateurs fonctionnaient à une température inférieure à 5 deg C, et 66,7% à une température inférieure à 7 deg C. Peu de réfrigérateurs (7,3%) fonctionnaient à une température supérieure à 9 deg C. Aucune caractéristique des réfrigérateurs, mis à part leur type, ne pouvait avoir une incidence sur les températures ou sur la distribution de température, dans les réfrigérateurs étudies.
(Mots clés: réfrigérateurs domestiques; température; produit réfrigéré)

As a chilled product moves along the chill chain it becomes increasingly difficult to control and maintain its temperature. Temperatures of bulk packs of chilled produce in large store rooms are far less sensitive to small heat inputs than single consumer packs in transport or open display cases. Before 1 April 1991, when the new Food Hygiene (Amendment) Regulations 1990 were implemented, there were no regulations in the UK to cover the temperature of chilled foods during distribution and retail display. The regulations, which are fully implemented over a two-year period, divide the majority of chilled foods into two groups: one consisting of the most *Listeria*-sensitive foods will have a maximum temperature during storage, transport and display of 5°C, whilst other foods considered less sensitive will have to be maintained below 8°C. Consistent policing of this new legislation should substantially improve the bacterial quality of chilled food when it is purchased by the consumer.

Although this new legislation should ensure that food producers and retailers maintain acceptable product temperatures during the distribution chain, they lose control when the product leaves the retail store. After a chilled product is removed from a display cabinet it spends a period outside a refrigerated environment whilst it is carried around the store and then transported home. In the first section of this paper some limited data on the temperature increase that can be obtained in this period are presented together with data about the rate of cooling that is subsequently achieved when the food is placed in a domestic refrigerator.

In a recent survey taken in China[1] only 2.3% of domestic refrigerators were found to operate with a temperature range of less than 6°C within the storage compartment: 34.1% had differences of 8–12°C, 34.1% in the range 12–14°C and 29.5% differences greater than 14°C. No similar data have been located for the UK or Europe. As part of a more wide-ranging survey of the consumer handling of chilled food, commissioned by the Ministry of Agriculture, Fisheries and Food (MAFF)[2], the Research Centre has gathered data on temperature in domestic refrigerators. The survey population consisted of 252 households selected from the towns of Weston-super-Mare, Bridgwater and Taunton. The survey was divided into two parts: the first was taken between September and December 1989 and the second between February and May 1990; the aim being to reflect varying seasonal ambient weather conditions. Each part of the survey consisted of 126 households split evenly between the three towns. The second part of this paper reports data on domestic refrigerators, with other aspects of the survey being reported in a second paper[3].

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Table 1 Maximum temperature (°C) measured in products after being transported for 1 h in the boot of a car without protection or within a cooled insulated container
Tableau 1 *Températures maximales (deg C) mesurées sur les produits après 1 heure de trajet jusqu'au domicile, dans la malle d'un véhicule ou dans un conteneur isotherme*

Product	Unprotected	Cool box
Beef pie	24	7
Chicken sandwich	32	10
Cooked chicken	28	12
Minced beef	18	9
Prepared salad	29	14
Quiche	26	18
Sausage (raw)	28	15
Smoked ham	30	14
Trout	28	5
Brie cheese	28	11
Coleslaw	30	14
Lasagne	21	6
Pate	25	13
Prawns	37	14
Raw chicken	24	4
Sausage roll	28	12
Smoked salmon	38	18

Transport from retail store to domestic storage

Over a 3 d period two samples of 19 different types of chilled product were purchased from a large retail store and carried to a car parked in the adjoining car park where temperature sensors were inserted into each sample. The sensors were attached to a miniature data-logging system (Stick-On, ACR Systems Inc.). One sample from each product was placed in a pre-cooled insulated box containing eutectic ice packs and the second left loose in the boot of the car. The car was then driven back to the centre and the product removed and placed in a domestic refrigerator after a total journey time of one hour. The ambient temperature during the journeys ranged from 23 to 27°C.

Initial product temperatures measured when the food reached the car ranged from 4 to over 20°C. The high temperatures measured in the thin sliced products such as salmon and ham were most probably due to heat pick-up during the time spent in the shopping trolley. Temperatures in larger products were indicative of the average display temperature together with a slight increase in the trolley. The initial temperatures indicated that one group of chilled products including minced beef, ready meals, raw chicken and beef pies were displayed at approximately 5°C. A second group including sausage rolls, sandwiches, quiche and prepared salads were close to 10°C. It was surprising to find cooked chicken in the second group. Some product temperatures of samples placed in the boot rose to approaching 40°C (*Table 1*) during the one-hour car journey, whilst most of the samples placed in the insulated box cooled during the car journey, except for a few at the top of the box which remained at their initial temperature.

Thin sliced products, i.e. smoked salmon trout (*Figure 1a*), showed the highest temperature changes during transport. Thicker products like cooked chicken (*Figure 1b*) were less influenced. After being placed in the domestic refrigerator it required approximately 5 h before the temperature was reduced below 7°C (*Figure 1a* and *b*).

Predictions made by using a mathematical model that calculated bacterial growth from temperature–time relationships indicated that increases of up to two generations in bacterial numbers (*Table 2*) can occur during this transport and domestic-cooling phase. The model assumes that bacteria require a period to acclimatize to a change in temperature (the lag phase) and that no acclimatization had occurred during display. If this rather optimistic assumption is not made, then up to 4.2 doublings of *Pseudomonas* and growth of both *Salmonella*

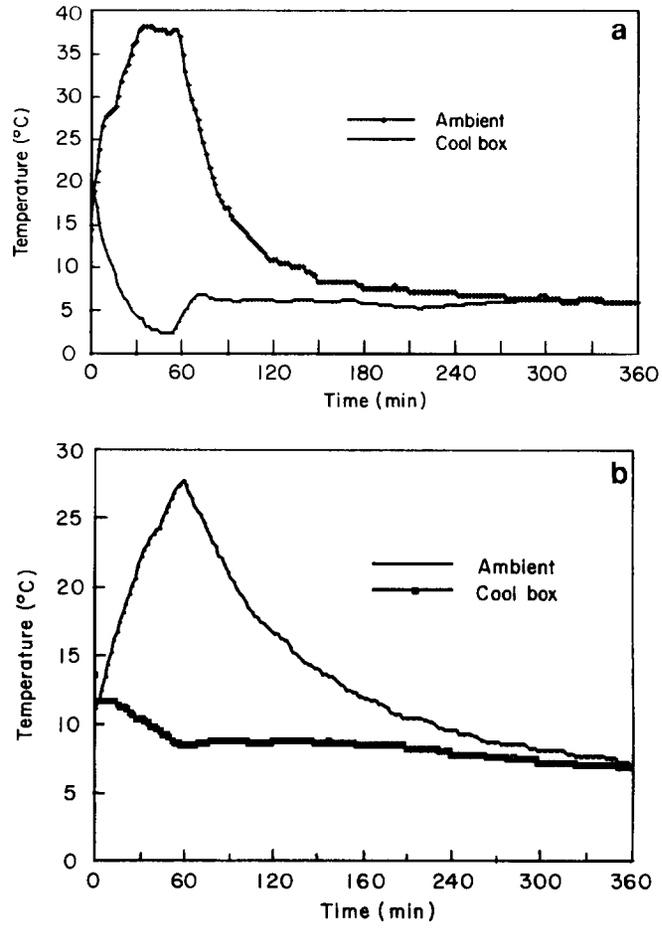

Figure 1 Temperature change in (a) smoked salmon trout and (b) Tandoori chicken during transportation home and being placed in a domestic refrigerator
Figure 1 *Changement de température dans (a) du saumon fumé et (b) du poulet tandoori, au cours du trajet jusqu'au domicile et dans le réfrigérateur domestique*

Table 2 Increase in bacterial numbers (generations) during 1 h in a car followed by 5 h in a domestic refrigerator
Tableau 2 *Augmentation du nombre de bactéries (en générations) pendant 1 heure de transport dans une voiture, suivie de 5 heures dans un réfrigérateur domestique*

Product	Conditions	*Pseudomonas*	*Clostridium*
Pate	Ambient	1.5	0.4
	Cool box	<0.4	0
Raw chicken	Ambient	1.6	0.2
	Cool box	0	0
Cooked chicken	Ambient	1.8	0.7
	Cool box	0	0
Prawns	Ambient	1.3	1.6
	Cool box	0	0
Brie cheese	Ambient	2.2	0.8
	Cool box	0	<0.1

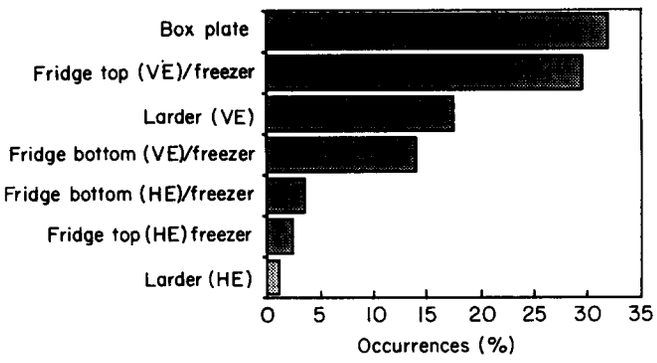

Figure 2 Percentage of different types of refrigerator found in survey: VE, visible evaporator; HE, hidden evaporator
Figure 2 *Pourcentage des différents types de réfrigérateurs considérés dans l'enquête: VE, évaporateur visible; HE, évaporateur caché*

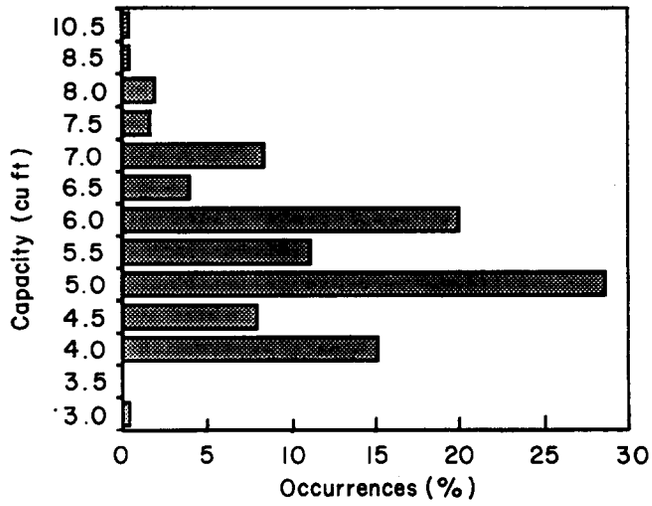

Figure 3 Percentage of different sizes of refrigerator found in survey
Figure 3 *Pourcentage des différentes dimensions des réfrigérateurs de l'enquête*

and *Listeria* were predicted. Very small increases in bacterial numbers (under 0.4 generations, *Table 2*) were predicted when the insulated box was used due to the lower product temperatures.

Within the home

In the initial part of the survey data were obtained on the refrigerators that were present. Only three of the 252 households surveyed did not have a working refrigerator. One of these owned a refrigerator that had recently stopped working and was planning to obtain a new model. One further person owned a refrigerator that was only used occasionally and was not in use during the survey. The most popular refrigerator design was a 'fridge–freezer' (49.4%), followed by the box-plate (31.9%) and larder refrigerators (18.7%). A more detailed breakdown of the types of refrigerator found in terms of refrigerator configuration and coil design is shown in *Figure 2*.

The capacity of the chilled food section of each refrigerator was visually assessed. Of refrigerators in the survey 82.9% were found to be between 4 and 6 cubic foot (cu ft) (1 cu ft $\approx$ 0.028 m^3). Less than 5% of the refrigerators were smaller than 3.5 cu ft or larger than 7.5 cu ft (*Figure 3*). Only three refrigerators (1.2%) had a fan mechanism in the chilled food section.

The age of each refrigerator was determined from information provided by the householder. A general assessment of the condition and type of refrigerator was also taken into account to ensure that the information was reasonably accurate. The majority of refrigerators (88%) were less than 10 years old. Over half (58.2%) were less than five years old and a small number (5.2%) were older than 20 years. Age differences were found to be affected by refrigerator type. Box-plate refrigerators were aged up to 31 years, whereas fridge–freezers were aged up to 18 years and larger refrigerators up to 10 years.

The appliance on the left- and right-hand side of each individual refrigerator was noted. Free standing was the most popular refrigerator position, which accounted for 13.6% of the results. When divided into the number of appliances with potential heat sources on either one or both sides, 25.5% of appliances had a potential heat source on one side and 1.2% had a potential heat source on both sides. Potential heat sources were dishwashers, freezers, ovens, tumble dryers and washing machines. Over half (59.8%) of householders positioned their refrigerators away from immediate heat sources, but with a unit or wall on either one or both sides.

The condition of the seals around the door of each refrigerator was judged subjectively on a scale from excellent to poor. Excellent was described as nearly new with good seal and no tears in the rubber. Seals that were poor were usually torn and perished and did not seal the door well. Of the refrigerator seals examined 60% were described as excellent or good and only 10% as poor. The seal condition was correlated with refrigerator age to determine whether poorer seals were found in older refrigerators. The resulting correlation was found to be relatively low (0.56), indicating that older refrigerators did not necessarily have worse seals. It is possible that some older refrigerators may have had seals replaced.

Mechanisms for setting refrigerator temperatures varied between different makes and models of refrigerator and therefore the refrigerator setting as a percentage of the full setting was recorded for each individual refrigerator. The greatest number of participants (21.8%) were found to set their refrigerators at between 41–50% of the maximum setting (*Figure 4*). Few participants set their refrigerators at less than 20% (7.3%) or greater than 80% (8.9%) of the full setting.

Only 15.1% of participants kept a thermometer in their refrigerator. One person owned a refrigerator with an integral thermometer that enabled temperatures to be read whilst the refrigerator door was closed. Thermometers were kept in the middle section of the refrigerator by 50% of participants with thermometers, and in the top by 23.7%. Only three people (1.2%) varied the thermometer position to measure temperatures in various parts of their refrigerator. If a thermometer was present the temperature reading was noted immediately after opening the refrigerator door. Under half (42.1%) of readings were below 5.9°C, with the greatest number of these being between 4 and 5.9°C. Five thermometers were either inaccurate or unreadable because of difficulty in finding the thermometer before ambient temperatures affected the reading.

Participants were asked to state the temperature at

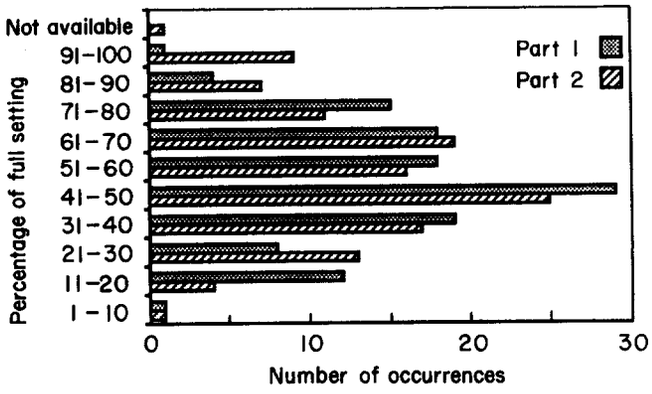

Figure 4 Temperature control settings on refrigerators
Figure 4 *Emplacements des capteurs de température dans les réfrigérateurs*

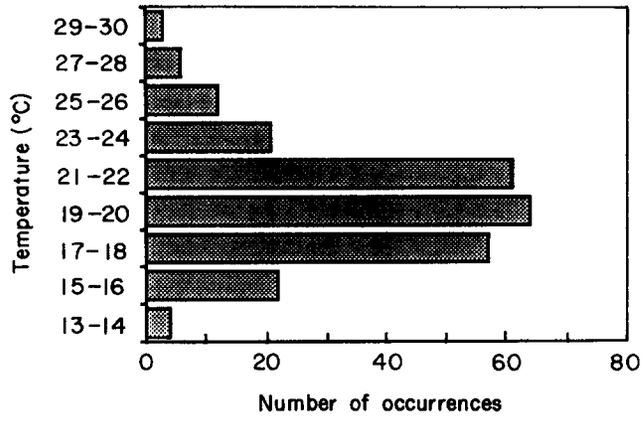

Figure 6 Ambient temperature near refrigerators
Figure 6 *Température ambiente près des réfrigérateurs*

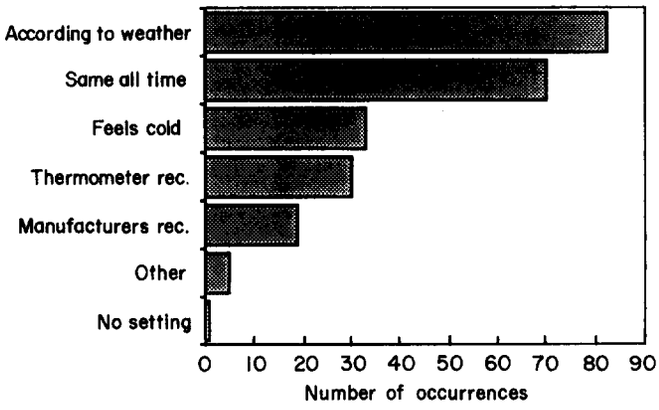

Figure 5 Methods used to set refrigerator temperature
Figure 5 *Méthodes utilisées pour fixer la température du réfrigérateur*

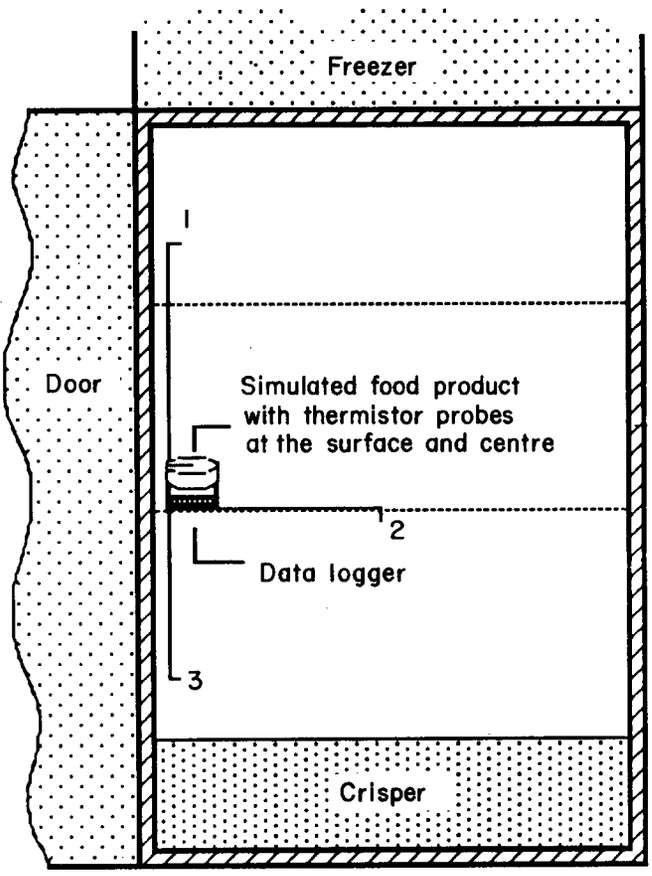

Figure 7 Positions of temperature sensors in refrigerators: 1, 2 and 3 are thermistors placed in air at top, middle and bottom of the refrigerator
Figure 7 *Emplacements des dispositifs de mesure de la température, dans les réfrigérateurs: 1, 2 et 3 sont des thermomètres placés dans l'air, en haut, au milieu et au bas du réfrigérateur*

which they tried to run their refrigerator. Nearly all participants were unable to name actual temperatures and gave answers based on the method actually used to set the temperature dial (*Figure 5*). A large number of people (32.8%) set their refrigerators according to the weather, setting the refrigerator to a lower temperature (higher setting) in the summer. It was interesting to note that although 38 participants had a thermometer in their refrigerator only 30 actually used the information to set their refrigerator temperature.

A spot reading of the ambient temperature near to each householder's refrigerator was recorded. The greatest number of people (72.2%) kept their houses at between 17 and 23°C, with an overall mean temperature of 20.6°C (*Figure 6*). Very few participants kept temperatures close to the refrigerator at less than 15°C (1.6%) or greater than 25°C (8.4%). Therefore, presuming that the spot readings recorded were representative of temperatures over a longer period, the majority of refrigerators would have been operating within the temperate zone conditions for which they were designed.

Finally, a miniature data-logger (Stick-On) with three air and two product sensors was placed into the refrigerator to monitor temperatures every 8 s and to record mean temperatures approximately every 5 min for a period exceeding 7 d. Air temperature sensors were positioned in the top, middle and bottom sections of the refrigerator. A simulated food product (87 mm diameter by 28 mm high disc of Tylose in a petri dish) was placed on the middle shelf. Sensors were placed in the geometric centre and centrally on the surface of the Tylose disc (*Figure 7*). After retrieving the loggers the data were

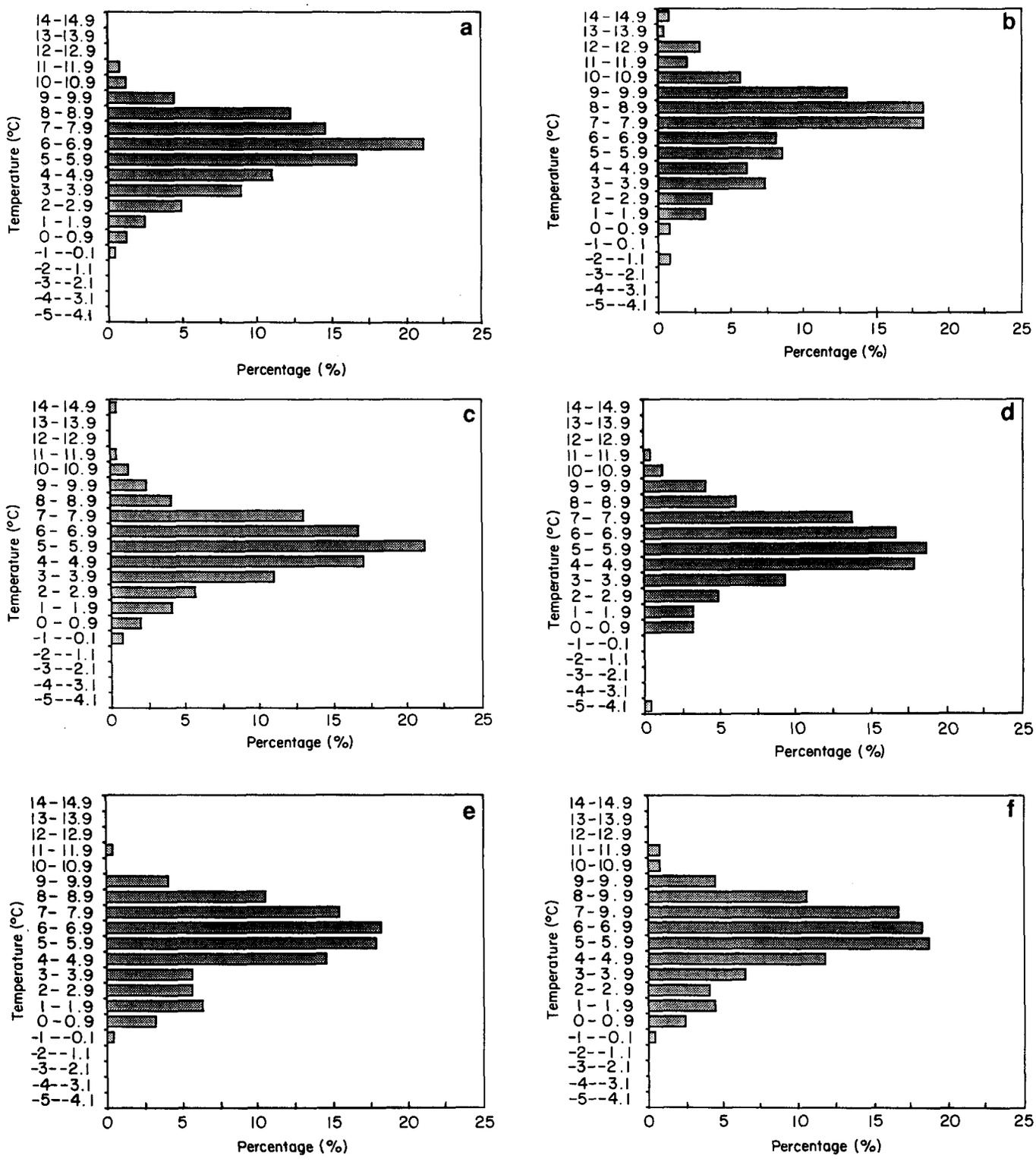

Figure 8 Percentage of refrigerators and the mean temperature measured at (a) overall, (b) top, (c) middle, (d) bottom, (e) product surface and (f) product centre

Figure 8 *Pourcentage des réfrigérateurs et température moyenne mesurée aux emplacements suivants: (a) dans tout le réfrigérateur; (b) en haut; (c) au milieu; (d) dans le bas; (e) à la surface du produit; et (f) à coeur du produit*

down-loaded onto an Amstrad 1640 PC before transfer to an Apple Macintosh, where the central 7 d temperature data were extracted for further analysis. Mean, minimum and maximum temperatures together with their standard deviations and cumulative frequency distribution were evaluated at each position in each refrigerator. Overall mean refrigerator temperatures were calculated from the above data.

The temperatures recorded in each individual refrigerator over the 7 d period were analysed and mean air temperatures calculated for the top, middle and bottom together with an overall air temperature for the whole refrigerator (*Figure 8a–d*). The product surface and product centre means were also calculated (*Figure 8e* and *f*). The highest recorded mean temperature was 11.4°C and the lowest −0.9°C, producing a range in mean tempera-

Table 3 Percentage of refrigerators with highest and lowest mean temperatures at top, middle or bottom
Tableau 3 *Pourcentage de réfrigérateurs enregistrant les températures moyennes les plus élevées et le plus basses, en haut, au milieu et au bas*

Position	Mean temperature	
	Highest	Lowest
Top	69.92	20.33
Middle	8.13	45.12
Bottom	21.95	34.55

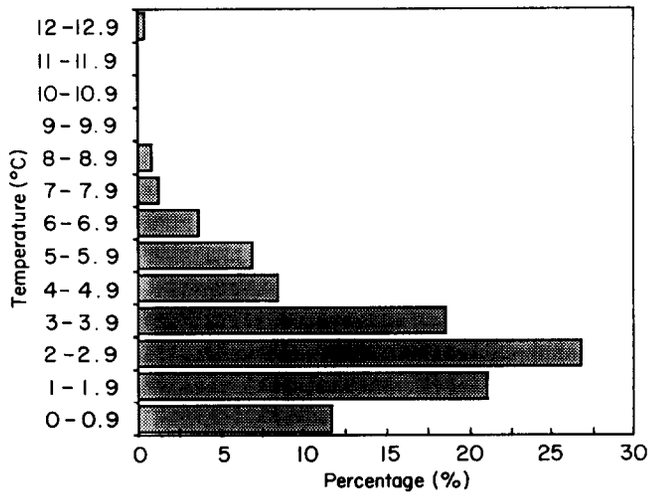

Figure 9 Difference between highest and lowest mean temperatures in refrigerators
Figure 9 *Différence entre les températures moyennes les plus élevées et les plus basses, dans les réfrigérateurs*

tures of 12.3°C. The overall mean air temperature for all the refrigerators in the survey was 6.04°C. In 69.9% of refrigerators the warmest place was in the top, and in 45.1% the coolest palce was in the middle (*Table 3*).

Analysis of variance of mean refrigerator temperatures revealed that overall refrigerator temperature and temperatures in the middle, bottom and at the simulated product surface and centre were not statistically different. The mean for all temperatures at the top of refrigerators was significantly higher ($P < 0.001$) than those for other positions.

In most refrigerators (86.9%) the range in temperatures between the highest and lowest mean was less than 5°C, with the greatest range being 12°C (*Figure 9*). However, if individual minimum and maximum temperatures were examined, much larger temperature ranges were calculated (*Figure 10*): the minimum range was 4.5°C and the largest 30.5°C (if recorded temperatures had not been averaged over 5 min periods these ranges may have been even greater).

Often the temperature at the top of a refrigerator is quoted as being approximately 5°C higher than temperatures at the bottom of a refrigerator. To test this theory the mean temperature at the bottom of all refrigerators examined was subtracted from the mean temperature at the top of each refrigerator. Only 6.9% of refrigerators

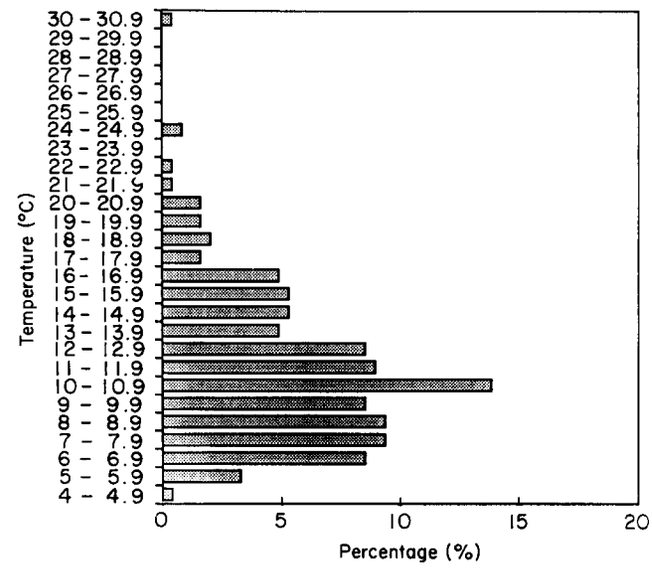

Figure 10 Difference between highest and lowest temperatures in refrigerators
Figure 10 *Différence entre les températures les plus élevées et les plus basses, dans les réfrigérateurs*

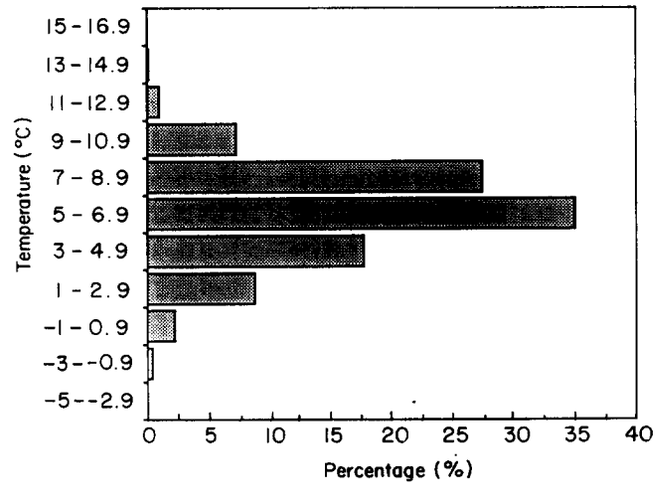

Figure 11 Percentage of time spent in different temperature ranges by average refrigerator
Figure 11 *Pourcentage du temps durant lequel un réfrigérateur moyen fonctionne à différentes plages de temperature*

fell within the 5–5.9°C category. A number of refrigerators (26.3%) were colder at the top than the bottom and therefore produced negative values. The minimum top-minus-bottom temperature found during the survey was −6.9°C and the maximum 12°C.

Frequency distribution of the percentage time spent between certain temperatures during the survey was calculated for all refrigerators. The greatest proportion of time (80.3%) was spent between 3 and 8.9°C (*Figure 11*). Only small amounts of time were spent above 9°C.

Only four refrigerators (1.6%) in the whole survey operated below 5°C during all the monitoring period and 33.3% of refrigerators spent all their time above 5°C. If divided into time spent below 5°C, 9.8% of refrigerators spent 90% or more of their time below 5°C and 27.4% of refrigerators spent 50% or more of their time below 5°C.

Analysis

Temperature data

Temperatures in each individual refrigerator monitored in the survey were analysed by analysis of variance (ANOVA) to determine whether a relationship existed between refrigerator temperature and refrigerator characteristics.

Mean temperatures over the whole refrigerator, in the top, middle and bottom sections and the product centre and surface were analysed against:

1. refrigerator type (box-plate, fridge-freezer, larder);
2. refrigerator setting (as percentage of full setting)
3. refrigerator size;
4. seal condition;
5. refrigerator age;
6. housing category of householder;
7. number per household;
8. age of householder;
9. ambient temperature in kitchen.

ANOVA revealed significant differences ($P < 0.001$) in refrigerator type and therefore refrigerator type was included in all further ANOVA. When analysed with refrigerator type all other refrigerator characteristics were not significant ($P > 0.05$). Therefore the only recorded refrigerator characteristic found to influence refrigerator temperatures was refrigerator type.

ANOVA revealed that box-plate refrigerators operated at lower temperatures than fridge–freezers or larder refrigerators ($P > 0.001$), which was due to fridge–freezers and larder refrigerators being warmer in the top section. Further analysis demonstrated that statistical differences only occurred between box-plate refrigerators and fridge–freezers and larder refrigerators with visible evaporators. Temperatures of all fridge–freezers and larder refrigerators were statistically similar.

The relationship between minimum temperature and refrigerator setting was investigated to determine whether the minimum recorded temperature was related to the base running temperature of refrigerators. Correlation between setting and minimum temperature was low (-0.247), indicating that the refrigerator setting could not be related to minimum temperatures.

Temperature distribution within refrigerator types was demonstrated by sorting top, middle and bottom temperatures into ascending temperature order. Results revealed that all larder refrigerators and 84.6% of fridge–freezers were warmest in the top section. Box-plate refrigerators were warmest in the bottom section in 59.7% of cases.

Further analysis of temperature distribution within refrigerator types revealed that statistical differences only occurred in fridge–freezers with a top refrigerator and visible evaporator and larder refrigerators with visible evaporators. The top sections of these refrigerators were significantly warmer than all other refrigerator types and positions.

Range in mean refrigerator temperatures

The range in mean temperatures was examined to determine the temperature variability within each type of appliance. The range varied from a minimum of 0.1°C to a maximum of 12°C with a mean range for all refrigerators of 2.9°C.

When refrigerators were examined by type a significant difference ($P < 0.001$) between types was found. Box-plate refrigerators operated over a narrower range of temperatures (mean range 1.8°C) than fridge–freezers (mean range 3.4°C) and larder refrigerators (mean range 3.7°C).

Discussion and conclusions

This study has looked at the two aspects of the chill chain that immediately precede the final preparation and consumption of the chilled food, the transportation of the food from retail to domestic storage and domestic refrigeration.

It is clear from the results presented in the first section that the temperature of chilled foods can rise to unacceptably high values if transported, without insulation, in a car boot. These data were obtained in June 1989, a very sunny period, but higher ambient temperatures are not uncommon in mid-summer. The predictions made show that substantial increases in bacterial numbers can occur during transportation and subsequent recooling. It is not difficult to think of even worse situations where chilled products are kept in the open backs of estate cars for many hours on hot summer days. However, a combination of increased consumer education and the use of insulated/pre-cooled containers should solve this particular problem. One refrigerator manufacturer is already advertising[4] that insulated bags are provided with their appliance to reduce temperature rises during transportation.

The basic design of domestic refrigerators has not changed in the last fifty years although their use and lately the type, complexity and microbiological sensitivity of the foods stored in them has changed markedly. Designers have responded to market demands for more compact appliances and more features, e.g. chilled drink and ice dispensers, but temperature control is only advertised as a sales point on more expensive multi-compartment refrigerators. Consumers now purchase and store a wide range of ready meals and other chilled products and they have demanded and obtained substantial reductions, and in some cases the total elimination, of preservatives and additives in these products. New chilled products are, therefore, inherently more bacterially sensitive and require closer temperature control than their predecessors. The introduction of new food temperature legislation should result in improved temperature control to the point of retail sale. If current predictions that eating habits will change from the current pattern of set meals to irregular frequent consumption are fulfilled, then the consequence is likely to be a demand for and purchase of more pre-prepared chilled foods and more visits to domestic refrigerators.

The refrigerators investigated in the survey were found to have an overall mean temperature of approximately 6°C in a temperature range from 11.4 to −0.9°C. Temperature ranges over the whole refrigerator varied from 4.5 to 30.5°C, with 3.7% of these being warmer than 20°C. On average 29.9% of refrigerators operated below 5°C and 66.7% operated below 7°C. Few refrigerators (7.3%) ran on average above 9°C. However, certain positions in the refrigerators ran at warmer temperatures

with 24.8% of positions at the top being above 9°C and an average temperature of 31.6°C being recorded in the middle of one refrigerator for 5 min of the monitoring period.

Overall the warmest temperatures were found in the top sections with temperatures in all other sections being statistically similar. Further analysis revealed that statistical differences in temperature distribution only occurred in fridge–freezers (with refrigerators at the top) and larder refrigerators, both with visible evaporators. The top part of 84.6% of fridge–freezers and all larder refrigerators were warmest. Box-plate refrigerators were found to have the lowest overall mean temperatures and the most even temperature distributions.

No refrigerator characteristic (apart from type) could be related to temperatures or temperature distribution in the refrigerators investigated. The results indicated that overall there was little difference between refrigerator settings, sizes, seal conditions or ages, but this may be due to the limited number of replicas in some categories. Few participants set their refrigerators at less than 20% or greater than 80% of the full dial setting and few refrigerators were below 4 cu ft or above 7 cu ft. Only 5.2% of refrigerators were more than 20 years old and only a small number (10%) had poor seals. The results show that temperatures in older refrigerators of a particular type were no worse than new ones of the same type. This indicates that changes in refrigerator design and control have had little effect on mean temperatures over the past 30 years.

Refrigerator temperatures were not related to ambient temperature. This was rather surprising because ambient temperatures are known to affect performance and are part of the BSI[5] (British Standards Institution) and ISO[6] (International Standards Organization) standard tests. The result may have been influenced by the data, which were mainly clustered between 17 and 23°C (72.2% of results), a range that may not have been large enough to affect significantly refrigerator temperature.

The social status and age of the householder and number of people per household were also found to be independent of refrigerator temperature. There is therefore no real evidence to suggest that any particular social or age group achieved better refrigerator temperature control. The number of people in households might be expected to affect refrigerator temperatures because in large households the loading and use would probably be greater. However, the results may have been influenced by the small number of very small (one member) or large (over five members) households (households with 2–4 members accounted for 83.3% of the results).

In laboratories, research has shown that refrigerator design and temperature setting affect temperature control and distribution. Factors such as loading patterns, loading of warm food and door openings are also known to influence refrigerator temperatures[7]. Further work is required to determine their influence in a practical situation.

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Behavior of *Listeria monocytogenes* at 7 °C in commercial turkey breast, with or without antimicrobials, after simulated contamination for manufacturing, retail and consumer settings

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Abstract

Uncured turkey breast, commercially available with or without a mixture of potassium lactate and sodium diacetate, was sliced, inoculated with a 10-strain composite of *Listeria monocytogenes*, vacuum-packaged, and stored at 4 °C, to simulate contamination after a lethal processing step at the plant. At 5, 15, 25 and 50 days of storage, packages were opened, slices were tested, and bags with remaining slices were reclosed with rubber bands; this simulated home use of plant-sliced and -packaged product. At the same above time intervals, portions of original product (stored at 4 °C in original processing bags) were sliced and inoculated as above, and packaged in delicatessen bags, simulating contamination during slicing/handling at retail or home. Both sets of bags were stored aerobically at 7 °C for 12 days to simulate home storage. *L. monocytogenes* populations were lower ($P<0.05$) during storage in turkey breast containing a combination of lactate and diacetate compared to product without antimicrobials under both contamination scenarios. Due to prolific growth of the pathogen under the plant-contamination scenario in product without lactate-diacetate during vacuum-packaged storage (4 °C), populations at 3 days of aerobic storage (7 °C) of such product ranged from 4.6 to 7.4 log cfu/cm^2. Under the retail/home-contamination scenario, mean growth rates (log cfu/cm^2/day) of the organism during aerobic storage ranged from 0.14 to 0.16, and from 0.25 to 0.51, in product with and without lactate-diacetate, respectively; growth rates in turkey breast without antimicrobials decreased ($P<0.05$) with age of the product. Overall, product without antimicrobials inoculated to simulate plant-contamination and product with lactate-diacetate inoculated to simulate retail/home-contamination were associated with the highest and lowest pathogen levels during aerobic storage at 7 °C, respectively. However, 5- and 15-day-old turkey breast without lactate-diacetate stored aerobically for 12 days resulted in similar pathogen levels (7.3–7.7 log cfu/cm^2), irrespective of contamination scenario.

Keywords: *Listeria monocytogenes*; Turkey breast; Antimicrobials

1. Introduction

Post-lethality treatment contamination of ready-to-eat (RTE) meat and poultry products with *Listeria monocytogenes* is a significant public health concern, as demonstrated by recent major listeriosis outbreaks (CDC, 1999, 2000, 2002) and risk assessment data (USDHHS-FDA-CFSAN/USDA-FSIS, 2003). Cross-contamination of RTE foods with the pathogen may occur both at the production and retail levels (Aguado et al., 2001; Van Coillie et al., 2004). Isolation of *L. monocytogenes* from a turkey processing plant, products, and cases of human listeriosis in Denmark demonstrated that end product contamination of turkey meat may pose high risk of infection for susceptible individuals (Ojeniyi et al., 2000). Molecular subtyping of food (prepared and handled in retail premises), environmental, and clinical isolates of *L. monocytogenes* in New York State, revealed that specific ribotypes of the organism, including ribotypes that have been linked to human disease, persisted over time in retail establishments (Sauders et al., 2004).

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Investigation of a large listeriosis outbreak in France, in 1992, linked to consumption of cooked pork tongue-in-jelly indicated that cross-contamination of other RTE products in delicatessen counters may have contributed to the magnitude of that particular outbreak (Jacquet et al., 1995). The potential of slicers and other utensils used in retail premises to serve as vehicles of cross-contamination of RTE meat and poultry products with *L. monocytogenes* also has been demonstrated (Humphrey and Worthington, 1990; Hudson and Mott, 1993; Lin et al., 2006; Vorst et al., 2006). Lin et al. (2006) reported that the transfer of the organism from an artificially contaminated slicer to deli meats was product-dependent, and more positive samples containing higher pathogen levels during storage at 4 °C were obtained for uncured oven-roasted turkey than for bologna containing potassium lactate and sodium diacetate or salami.

Numerous studies have been conducted in different countries to determine the prevalence and levels of *L. monocytogenes* in retail RTE products, and various incidence rates have been reported (Wilson, 1995; Uyttendaele et al., 1999; Aguado et al., 2001; Gombas et al., 2003; Van Coillie et al., 2004; Vitas et al., 2004; Angelidis and Koutsoumanis, 2006). Gombas et al. (2003) examined RTE foods collected from retail markets in the United States, and reported a *L. monocytogenes* prevalence of 0.89% in sliced luncheon meats. The observed trend for in-store-packaged luncheon meats, and deli and seafood salads to have higher incidence of the pathogen than manufacturer-packaged products, as reported by these investigators, may be regarded as indicative of the potential contribution of handling of RTE products at retail to their contamination with the organism. In addition to food-processing and retail establishments, the domestic environment and consumer food-handling practices also have been acknowledged as important risk factors for foodborne listeriosis (ILSI Research Foundation/Risk Science Institute, 2005; Yang et al., 2006). According to a recent consumer phase risk assessment, in which the data collected by Gombas et al. (2003) were used as initial contamination levels, approximately 0.3% of servings of deli meats were predicted to be contaminated with levels of *L. monocytogenes* higher than 10^4 cfu/serving at the time of consumption (Yang et al., 2006).

Risk assessment data indicate that control measures that prevent occurrence of high levels of *L. monocytogenes* at the point of consumption are expected to have the greatest impact on reducing the incidence of listeriosis (Nørrung, 2000; Chen et al., 2003). In order to prevent high levels of the pathogen in products that support its growth, information associated with its behavior in such products under common handling and storage conditions, preceding consumption, is required. Nevertheless, the majority of research studies have evaluated the behavior of *L. monocytogenes* or the efficacy of antilisterial hurdles during vacuum-packaged storage of RTE foods, and data on the fate of the pathogen under handling and storage conditions encountered at retail or in the home are limited.

This study was undertaken to evaluate the behavior of *L. monocytogenes* in uncured RTE turkey breast, commercially available with or without a mixture of potassium lactate and sodium diacetate, during aerobic storage at 7 °C under two contamination scenarios. The objective was to assess the levels of the organism to which consumers could be potentially exposed during home storage of contaminated product. Product was inoculated under conditions chosen to simulate contamination at production, or following storage and subsequent slicing/handling at the retail or home level. Product inoculated under each of the two contamination scenarios was stored aerobically at 7 °C for 12 days to simulate domestic storage conditions prior to consumption. Growth trends of the spoilage microflora of the product under the above conditions also were assessed.

2. Materials and methods

2.1. *L. monocytogenes* strains

A 10-strain composite was used in this study and included strains 558 (serotype 1/2, pork meat isolate), NA-1 (serotype 3b, pork sausage isolate), N-7150 (serotype 3a, meat isolate), N1-225 and N1-227 (serotype 4b, clinical and food isolates, respectively, associated with the same outbreak; CDC, 1999), R2-500 and R2-501 (serotype 4b, food and clinical isolates, respectively, associated with the same outbreak; CDC, 2001), and R2-763, R2-764 and R2-765 (serotype 4b, clinical, food and environmental isolates, respectively, associated with the same outbreak; CDC, 2002). Strains N1-225, N1-227, R2-500, R2-501, R2-763, R2-764 and R2-765 were kindly provided by Dr. Martin Wiedmann (Cornell University, Ithaca, New York). Selection of strains was based on their growth behavior in culture broth, as evaluated in a previous study conducted in our laboratory (Lianou et al., 2006), as well as on their origin. Strains with robust growth characteristics at 4 °C and outbreak-related isolates were primarily chosen, in compliance with recommendations of the National Advisory Committee on Microbiological Criteria for Foods (NACMCF, 2005). All strains were available as refrigerated (4 °C) cultures on tryptic soy agar (TSA; Difco, Becton Dickinson, Sparks, Maryland) supplemented with 0.6% yeast extract (YE; Acumedia, Baltimore, Maryland) slants, and working cultures were stored in this form and subcultured monthly. Strains were activated by transferring a single colony from PALCAM agar (Difco, Becton Dickinson) plates into 10 ml of tryptic soy broth (TSB; Difco, Becton Dickinson) supplemented with 0.6% YE, and incubating at 30 °C for 24 h. Activated cultures were subcultured (0.1 ml) into 10 ml of fresh TSBYE and incubated at 30 °C for 24 h.

2.2. Inoculum preparation

For inoculum preparation, 24-h cultures of each of the strains were centrifuged and washed separately as described in previous studies (Geornaras et al., 2005, 2006). The harvested cells were resuspended in 10 ml of turkey breast extract (each culture separately) and stored at 7 °C for approximately 3 days (60–72 h), to allow for acclimatization of the cells to a low-temperature food environment. The turkey breast extract was prepared from frozen (−20 °C) portions of product without potassium lactate–sodium diacetate. To prepare the extract, turkey breast was thawed (4 °C) overnight and then homogenized (Masticator, IUL Instruments, Barcelona, Spain) with distilled water for 2 min to yield a 10% (w/w) suspension of the product, which was subsequently passed twice through cheesecloth, autoclaved for 15 min and cooled to ambient temperature (25 °C) before use. The pH of product extract used for inoculum preparation was measured both before (6.39 ± 0.07) and after (6.40 ± 0.07) autoclaving using a digital pH meter with a glass electrode (Denver Instruments, Arvada, Colorado).

Following the acclimatization period, the cultures were combined and serially diluted in freshly prepared product extract to yield an inoculum level of 1–2 log cfu/cm^2 when 0.1 ml of inoculum was applied to each side of a piece of sliced turkey breast.

2.3. Product inoculation

Unfrozen turkey breast without or with potassium lactate (1.5%) and sodium diacetate (0.05%) was obtained from a commercial manufacturer and used within 5 days of production. The formulation of product without antimicrobials consisted of turkey breast, turkey broth, salt, modified food starch, sugar, carrageenan, sodium phosphate and flavor. The formulation of product containing antimicrobials consisted of turkey breast, water, potassium lactate, salt, sugar, carrageenan, sodium phosphate, sodium diacetate, and turkey flavor (turkey stock, modified food starch, salt, flavor). The fat and moisture contents of the product were determined according to the AOAC International official methods (960.39 and 950.46.B, respectively; AOAC, 1998). The fat content (%) of product with and without antimicrobials was 1.0 ± 0.3 and 0.9 ± 0.3, while the moisture content (%) was 76.2 ± 6.6 and 76.8 ± 0.6, respectively. Before inoculation, turkey breast was sliced, and slices (approximately 3 mm thick) were then cut into pieces (5 × 5 cm, 25 cm^2 per side). Pieces were inoculated under a biological safety cabinet as described by Geornaras et al. (2005).

Twelve pieces of inoculated product were stacked on top of each other and placed in vacuum bags (20 × 25 cm, 3 mil std barrier, Nylon/PE vacuum pouch, Koch, Kansas City, Missouri), vacuum-packaged (Hollymatic Corp., Countryside, Illinois), and stored at 4 °C, to simulate post-lethality contamination at the processing plant. At 5, 15, 25 and 50 days of storage, packages were opened, pieces were tested (microbiological and chemical analyses), and bags with remaining pieces were reclosed by folding with rubber bands; this simulated purchase and domestic use of plant-sliced and -packaged product. Additionally, at the same above time intervals, portions of original unsliced product (stored at 4 °C in original processing vacuum packages) were sliced, cut, and inoculated as above, and 12 pieces of product stacked on top of each other were placed on delicatessen paper (20 × 27 cm, Glenvale Deli Wrap, Dixie Food Service, Georgia-Pacific, Atlanta, Georgia) and packaged in reclosable delicatessen bags (20 × 25 cm, Koch), simulating contamination during slicing/handling at retail or at home. Both sets of samples (i.e., vacuum bags that were opened and reclosed with rubber bands, and delicatessen bags) were left at ambient temperature (25 °C) for 90 min (Audits International, 1999; Kennedy et al., 2005) to simulate temperature abuse of the product (e.g., time lapse between purchase and home refrigeration, consumer handling, etc.). Bags were then stored at 7 °C for 12 days (Fig. 1). The latter storage conditions (i.e., aerobic storage at mildly abusive temperature) were applied to simulate home storage of pre-packaged, or sliced and packaged at retail, RTE turkey breast (NACMCF, 2005).

2.4. Microbiological analyses

Samples were analyzed on days 0, 5, 15, 25 and 50 of refrigerated (4 °C) vacuum-packaged storage, and on days 0, 3, 6, 9 and 12 of aerobic storage (7 °C) following 5, 15, 25 and 50 days of vacuum-packaged storage of the product (either sliced and inoculated, or unsliced; Fig. 1). Two consecutive pieces from each bag were transferred aseptically to a sterile 24 oz bag (Whirl-Pak®, Nasco, Modesto, California) containing 50 ml of maximum recovery diluent (MRD; 0.85% NaCl and 0.1% peptone), and shaken vertically 30 times (Samelis et al., 2001; Barmpalia et al., 2004). Appropriate serial dilutions in 0.1% buffered peptone water (Difco, Becton Dickinson) were surface plated on the following media: TSAYE for the enumeration of total mesophilic and total psychrotrophic microbial populations; PALCAM agar for the enumeration of *L. monocytogenes*; and Rose Bengal Chloramphenicol agar (RBC; Difco, Becton Dickinson) for the enumeration of yeasts and molds. Moreover, presumptive lactic acid bacteria (LAB) were enumerated by pour-plating in de Man Rogosa Sharpe agar (MRS; Biotrace International Inc., Bothell, Washington) as described in previous studies (Barmpalia et al., 2004, 2005). Colonies were counted after incubation at 25 °C for 72 h (TSAYE-total mesophiles, MRS) or 7 days (RBC), at 30 °C for 48 h (PALCAM), or at 7 °C for 14 days (TSAYE-total psychrotrophs).

2.5. pH and water activity measurements

Each sample, after being plated, was homogenized (Masticator, IUL Instruments) for 2 min, and the pH of the homogenate was measured using a digital pH meter

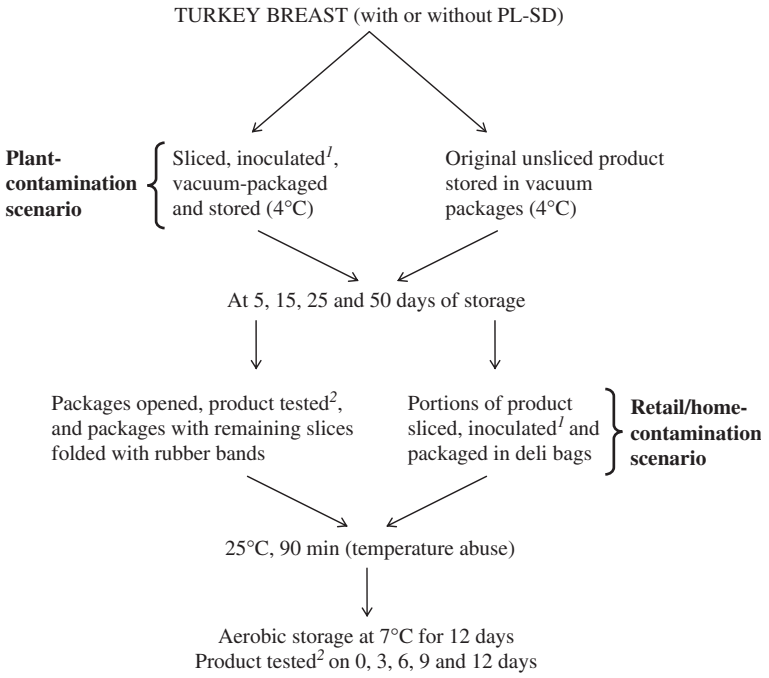

Fig. 1. Inoculation of turkey breast with *Listeria monocytogenes* under conditions simulating contamination at plant or retail/home, and storage under simulated home storage conditions. PL: potassium lactate; SD: sodium diacetate; [1]10-strain composite of *L. monocytogenes* (1–2 log cfu/cm^2); [2]microbiological and chemical analyses.

with a glass electrode (Denver Instruments). Furthermore, at 0, 5, 15, 25 and 50 days of vacuum-packaged storage (4 °C), a turkey breast piece was transferred to a Whirl-Pak® bag containing distilled water (1:10), homogenized for 2 min, followed by pH measurement as described above. Water activity (a_w) of turkey breast was measured during vacuum-packaged storage using an AquaLab (model series 3, Decagon Devices Inc., Pullman, Washington) water activity meter (Georgnaras et al., 2005, 2006).

2.6. Data analysis

The experiment was conducted twice with different product batches and, for each replication, three individual samples were analyzed at each sampling time ($n = 6$). Microbiological data were converted to log cfu/cm^2 and, for samples inoculated to simulate retail/home-contamination, growth rates during aerobic storage (7 °C) were determined using the linear regression function of Microsoft Excel. Data were analyzed using the mixed model procedure of SAS® (SAS, 2002). Means and standard deviations were calculated and least-squares means were separated using the pairwise *t*-test at $\alpha = 0.05$.

3. Results and discussion

3.1. L. monocytogenes populations under the plant-contamination scenario

In turkey breast without lactate-diacetate, *L. monocytogenes* populations (log cfu/cm^2) increased from 1.6 on day-0 to 7.3 on day-25 of storage in vacuum packages (4 °C), and approximately the same levels were observed on day-50 (Fig. 2). Extensive growth of *L. monocytogenes* in processed poultry products formulated without antimicrobial compounds and stored at refrigeration or abusive temperatures has been demonstrated in numerous investigations (Glass and Doyle, 1989; Wederquist et al., 1994; Beumer et al., 1996; Islam et al., 2002; Zhu et al., 2005). In addition to the favorable pH and a_w conditions for microbial growth in poultry products, the profuse growth of the organism in turkey breast in the present study may be attributed to the fact that this particular product was uncured. Inhibition of growth of *L. monocytogenes* exerted by curing ingredients such as sodium nitrite, as well as lower prevalence of the organism in cured than in uncured meat products have been documented (Grau and Vanderlinde, 1992; Duffy et al., 1994; Farber and Daley, 1994; Vitas et al., 2004).

In turkey breast with lactate-diacetate, *L. monocytogenes* populations were lower ($P < 0.05$) compared to those on product without antimicrobials at each sampling period during vacuum-packaged storage (4 °C), and increased from 1.7 log cfu/cm^2 on day-0 to 5.3 log cfu/cm^2 on day-50 of storage (Fig. 2). The antilisterial efficacy of potassium or sodium salts of lactic acid and sodium acetate or diacetate used as formulation ingredients in RTE meat products, either individually or in combination, has been demonstrated by numerous studies (Bedie et al., 2001; Mbandi and Shelef, 2002; Samelis et al., 2002; Seman et al., 2002; Stekelenburg, 2003; Barmpalia et al., 2004, 2005; Georgnaras et al., 2006), and by relatively fewer investigations in

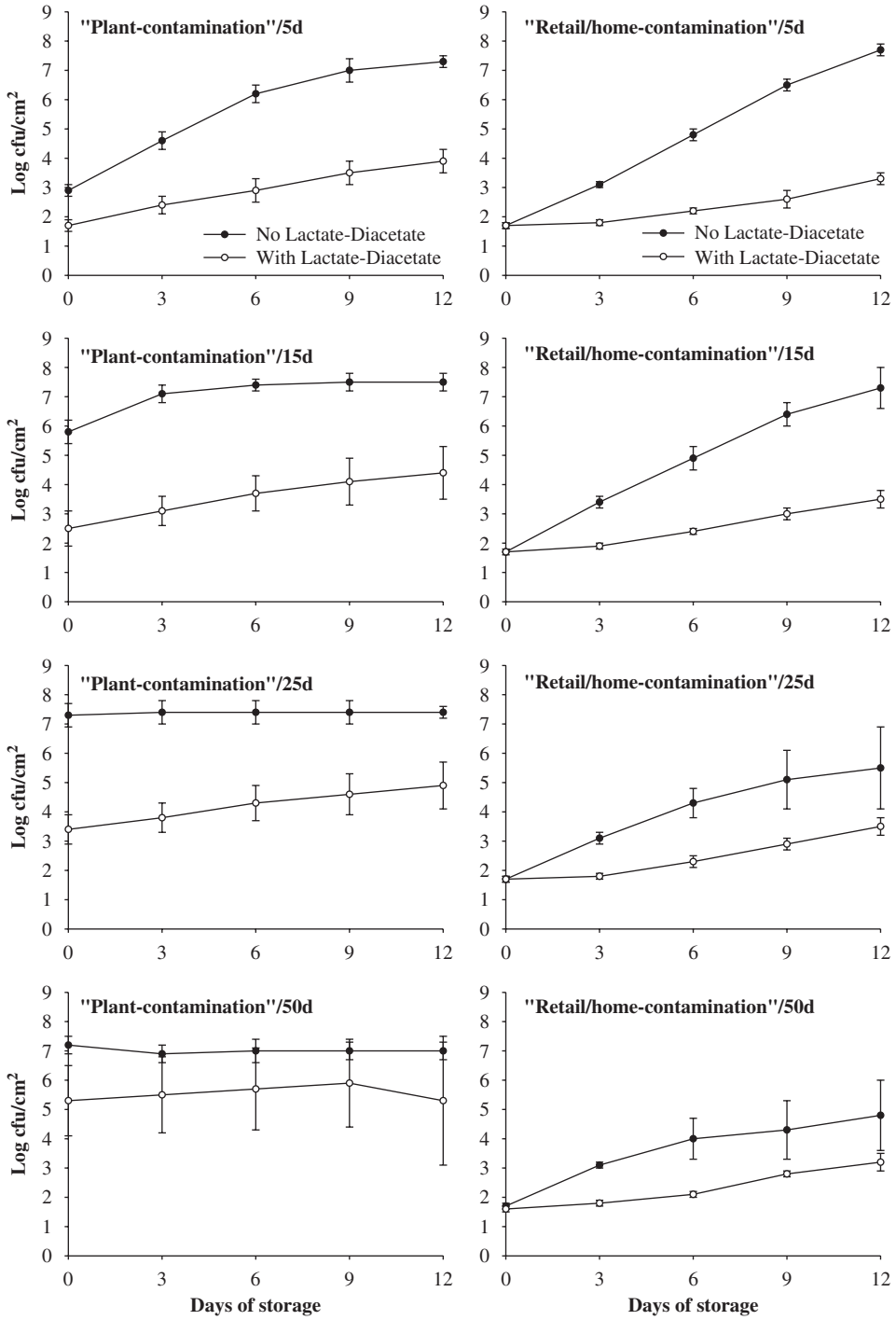

Fig. 2. Mean populations (log cfu/cm^2 ± standard deviation, $n = 6$) of *Listeria monocytogenes* (PALCAM agar) on turkey breast slices with or without potassium lactate–sodium diacetate stored aerobically at 7 °C for 12 days, (i) at 5, 15, 25 and 50 days of vacuum-packaged storage at 4 °C following inoculation (1–2 log cfu/cm^2) with the pathogen (''Plant-contamination''), or (ii) sliced and inoculated (1–2 log cfu/cm^2) with the pathogen after 5, 15, 25 and 50 days of vacuum-packaged storage of unsliced product at 4 °C (''Retail/home-contamination'').

processed poultry products (Schlyter et al., 1993a, b; Wederquist et al., 1994; Zhu et al., 2005).

As expected, the fate of *L. monocytogenes* during aerobic storage following the plant-contamination scenario was affected by its behavior during the preceding vacuum-packaged storage. Pathogen populations attained at 5, 15, 25 and 50 days of vacuum-packaged storage of the product at 4 °C constituted the day-0 counts of aerobic storage at 7 °C (Fig. 2). Due to the prolific growth of the organism in turkey breast without lactate-diacetate during storage in vacuum packages, pathogen populations attained within 3 days of subsequent aerobic storage ranged from 4.6 to 7.4 log cfu/cm^2; in product packages that were opened at 25 and 50 days of vacuum storage, pathogen levels of

approximately 7.0 log cfu/cm^2 were already present on day-0 of aerobic storage (Fig. 2). Since the infectious dose for *L. monocytogenes* is strongly associated with parameters such as the food matrix, strain virulence and host susceptibility (McLauchlin et al., 2004), relatively low doses cannot be excluded from causing infection to high-risk population groups (Farber and Peterkin, 1991). With *L. monocytogenes* growth to a level observed in this study, post-processing plant-contamination of uncured turkey breast without antimicrobials may result in potentially hazardous pathogen levels within 0–3 days of subsequent home storage of opened packages. Although shelf life is usually a quality measurement, the above observations indicate that establishing safety-oriented "sell-by" or "use-by" dates by processors of uncured poultry products should be also considered when no antilisterial interventions are in place. Public health concerns associated with extended shelf life of certain foods, as well as the potential value of safety-based date labeling for the control of *L. monocytogenes* in refrigerated RTE products, have been acknowledged (NACMCF, 2005).

In turkey breast containing lactate-diacetate, pathogen populations at 12 days of aerobic storage ranged from 3.9 to 5.3 log cfu/cm^2 with increasing age of the product (length of previous refrigerated vacuum-packaged storage). On the one hand, in 5- and 15-day-old product, pathogen levels of approximately 3 log cfu/cm^2 were reached within 6 and 3 days of aerobic storage, respectively, while populations of approximately 4 log cfu/cm^2 were observed within 12 and 9 days, respectively. On the other hand, slower growth of the pathogen was observed in 25-day-old product, while in 50-day-old product, although initial pathogen levels were 5.3 log cfu/cm^2, no further growth ($P \geqslant 0.05$) was observed during the 12-day storage period (Fig. 2). The latter observation could be attributed to the high levels of spoilage microflora encountered during vacuum-packaged, and consequently, aerobic storage of turkey breast with lactate-diacetate, as demonstrated by the comparative evaluation of the counts obtained on PALCAM agar and TSAYE (Figs. 2 and 3). Although lower ($P < 0.05$) *L. monocytogenes* populations were observed in turkey breast containing lactate-diacetate compared to product without antimicrobials, the observations made here indicate that these antimicrobial compounds, depending on concentrations applied (USDA-FSIS, 2000), may not be sufficient to completely inhibit growth of the organism. Therefore, consumer exposure to potentially hazardous levels of the pathogen may not be avoided; such levels under this contamination scenario could be reached either due to prolonged shelf life of the product, or due to extended home storage of opened product packages by the consumers (Fig. 2).

3.2. *L. monocytogenes* populations under the retail/home-contamination scenario

Pathogen populations (log cfu/cm^2) increased from 1.6–1.7 on day-0 to 3.2–3.5 and 4.8–7.7 on day-12 of aerobic storage (7 °C) in product with and without lactate-diacetate, respectively (Fig. 2). In turkey breast without lactate-diacetate, significant ($P < 0.05$) increases in pathogen levels were observed within 3 days of aerobic storage, resulting in populations of 3.1–3.4 log cfu/cm^2. Lactate-diacetate inhibited ($P < 0.05$) growth of the organism, and populations of approximately 3 log cfu/cm^2 were reached only at 9 or 12 days of aerobic storage. Mean growth rates (log cfu/cm^2/day) of *L. monocytogenes* during aerobic storage of turkey breast of different age (length of vacuum-packaged storage of original product before slicing and inoculation) are shown in Fig. 4, and ranged from 0.14 to 0.16, and, from 0.25 to 0.51, in product with and without lactate-diacetate, respectively. Growth rates of the pathogen in turkey breast without antimicrobials decreased with product age: the organism grew slower ($P < 0.05$) in product that was sliced and inoculated after 25 and 50 days than in product sliced and inoculated after 5 and 15 days of vacuum-packaged storage (Fig. 4). This observation could be attributed to growth of spoilage microorganisms observed during storage (4 °C) in vacuum packages of original unsliced turkey breast without lactate-diacetate; total microbial counts on day-0 of aerobic storage of product sliced and inoculated with the pathogen at different points of its vacuum storage appeared to increase (Fig. 3).

Although, the growth potential of *L. monocytogenes* has been evaluated in various products and at different storage temperatures, there is limited information relative to the behavior of the organism under conditions similar to those encountered during handling and storage of foods at retail or in the home (Burnett et al., 2005; Yang et al., 2006). Burnett et al. (2005) reported that a 14-day aerobic storage period of turkey breast at 5, 7 or 10 °C (conditions chosen to simulate additional handling at retail) supported prolific growth of the pathogen. Growth rates of the organism (log cfu/g/day) were 0.45, 0.83 and 1.53 during storage at 5, 7 and 10 °C, respectively, and were higher than those observed for ham and cold-smoked salmon, particularly at 7 and 10 °C. As indicated by the data presented here, contamination of uncured turkey breast with *L. monocytogenes* during slicing/handling at retail or home could pose a health risk for susceptible individuals during home storage of the product. Nevertheless, this risk can be reduced considerably when antimicrobials are incorporated in the formulation of the product and when proper food-handling practices (i.e., short-term retail/home storage) are followed.

The "date marking" requirements of the 2005 Food Code, proposed as a means of controlling the temperature-time combination for retail cold holding of potentially hazardous RTE foods after opening of the package, allow for a maximum of 7 days of storage at 5 °C or 4 days at 7 °C. These cold holding provisions were developed so as not to allow 1 log of *L. monocytogenes* growth (USDHHS-PHS-FDA-CFSAN, 2005). Under the conditions of this study and under the retail/home-contamination scenario of

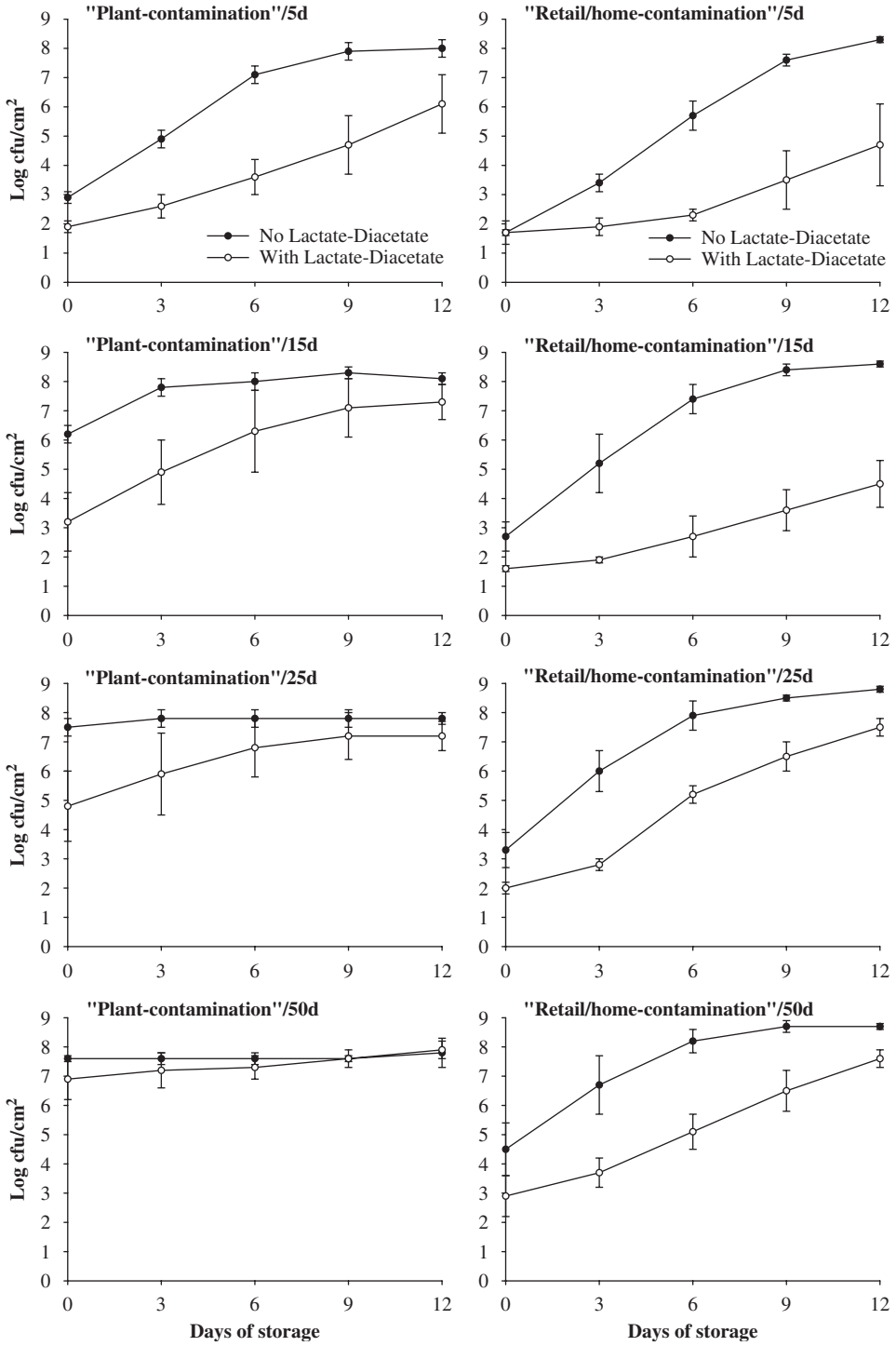

Fig. 3. Mean total psychrotrophic microbial populations ($\log$ cfu/cm$^2 \pm$ standard deviation, $n = 6$) (TSAYE) on turkey breast slices with or without potassium lactate–sodium diacetate stored aerobically at 7 °C for 12 days, (i) at 5, 15, 25 and 50 days of vacuum-packaged storage at 4 °C following inoculation (1–2 $\log$ cfu/cm^2) with *Listeria monocytogenes* (''Plant-contamination''), or (ii) sliced and inoculated (1–2 $\log$ cfu/cm^2) with *Listeria monocytogenes* after 5, 15, 25 and 50 days of vacuum-packaged storage of unsliced product at 4 °C (''Retail/home-contamination'').

turkey breast without lactate-diacetate, observed increases of the pathogen within 3 days of aerobic storage at 7 °C were >1 $\log$ cfu/cm^2. Hence, re-evaluation of the above requirements may be needed with respect to refrigerated retail storage of uncured poultry products without antimicrobials. According to the NACMCF, it might be necessary for safety-based date labeling to be applied at various points in the food chain; labeling at the consumer and food-handler level of the format ''use within *x* days'' of opening or purchase, appears to be very promising in controlling *L. monocytogenes* when coupled with good temperature control (NACMCF, 2005). Such a concept

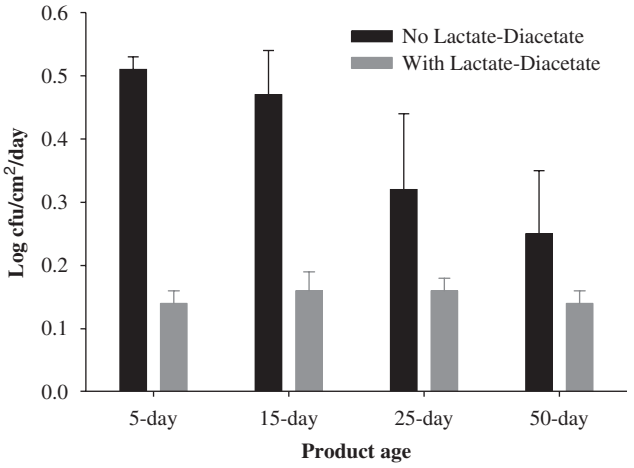

Fig. 4. Mean growth rates (log cfu/cm^2/day ± standard deviation, $n = 6$) of *Listeria monocytogenes* (PALCAM agar) on the surface of turkey breast with or without potassium lactate–sodium diacetate stored aerobically at 7 °C for 12 days, after being sliced and inoculated (1–2 log cfu/cm^2) with the pathogen at 5, 15, 25 and 50 days of vacuum-packaged storage of unsliced product at 4 °C.

can be particularly important in the case of RTE foods such as the one used in the present study, which, due to repeated use by the consumers followed by refrigerated storage, are highly susceptible to recontamination and growth of the pathogen.

Overall, product without antimicrobials inoculated to simulate plant-contamination and product containing lactate-diacetate inoculated to simulate retail/home-contamination were associated with the highest and lowest levels of *L. monocytogenes* during aerobic storage at 7 °C, respectively (Fig. 2). Due to pathogen growth observed both in product without and with (although at slower rate) lactate-diacetate during vacuum-packaged storage (4 °C), the plant-contamination scenario was generally associated with higher pathogen levels during aerobic storage (7 °C) than the retail/home-contamination scenario. Nevertheless, aerobic storage of 5- and 15-day-old product without antimicrobials for 12 days resulted in similar levels of the organism (7.3–7.7 log cfu/cm^2), irrespective of contamination scenario (Fig. 2). Data on storage times of RTE foods at retail or in the home, to be used in assessing the risk associated with growth of *L. monocytogenes* before consumption, are limited. According to the findings of a consumer-based study in Sweden, the majority of the participants reported that they stored opened packages of ham for 3 days to 1 week, but storage times as long as 2 weeks or based on the "best-before" date on the product label or on personal judgment also were reported (Marklinder et al., 2004). Based on the results of the present study, such practices may contribute to exposure of susceptible individuals to levels of *L. monocytogenes* able to cause infection. According to recent risk assessment findings, domestic food-handling practices can increase the mean mortality (death/serving) from consumption of deli meats for the intermediate-age US population (total population excluding elderly and pregnancy-associated groups) by as much as 10^6 times, and inadequate refrigeration temperature followed by storage time were determined to be the practices contributing the most to increased risk of listeriosis (Yang et al., 2006). However, the effect of storage temperature and time on mean mortality was different in different types of deli meats, with opened packages of deli meats demonstrating higher risk than unopened vacuum packages or fresh-sliced products.

Although the present study was only a simulation of contamination at the processing or retail/home level, the collected data indicate that home storage of plant-contaminated product may result in consumer exposure to similar or higher levels of the organism compared to product contaminated subsequently in the food chain, depending on application and efficacy of antilisterial interventions, product age and length of storage. A survey, undertaken to determine the prevalence and concentrations of *L. monocytogenes* in RTE foods collected from retail markets in Maryland and northern California, demonstrated a trend for manufacturer-packaged products to have higher levels of the organism than in-store-packaged products (Gombas et al., 2003). According to Yang et al. (2006), contamination level of *L. monocytogenes* in deli meats at retail represented the input parameter with the highest correlation with mortality in a consumer phase risk assessment approach. However, when assessing the relative risk of listeriosis associated with RTE meat or poultry products that are either purchased pre-sliced and pre-packaged or sliced and packaged at retail, prevalence of the pathogen, consumption data and consumer preferences also need to be considered. Gombas et al. (2003) reported that the incidence of *L. monocytogenes* tended to be higher in in-store-packaged luncheon meats (2.7%) than in manufacturer-packaged products (0.4%), supporting findings of additional investigations indicating the high likelihood of retail premises to serve as a source of cross-contamination of RTE products with the organism (Uyttendaele et al., 1999; Aguado et al., 2001; Van Coillie et al., 2004).

3.3. Spoilage microflora

The high similarity in total mesophilic (data not shown) and total psychrotrophic (Fig. 3) microbial counts obtained on TSAYE during storage in vacuum packages and under aerobic conditions, demonstrated that the spoilage microflora of the product was composed almost exclusively by psychrotrophs. Comparative evaluation of the counts obtained on PALCAM agar (Fig. 2), TSAYE (Fig. 3) and MRS agar (data not shown) illustrated growth of spoilage microflora, and primarily presumptive LAB, after 25 and 50 days of vacuum-packaged storage (4 °C) of product with and without lactate-diacetate, respectively. LAB bacteria, and primarily members of the genera *Lactobacillus* and *Leuconostoc*, constitute the major bacterial group associated with spoilage of refrigerated

vacuum-packaged processed meats (Borch et al., 1996; Samelis et al., 2000; Barmpalia et al., 2005). Although effective antilisterial treatments have been shown to provide inhibition against this bacterial group during refrigerated vacuum-packaged storage of RTE meats (Barmpalia et al., 2005), lactate-diacetate did not inhibit growth of presumptive LAB under the conditions of this study.

Changes in populations of presumptive LAB during aerobic storage (7 °C) were variable (data not shown), even within samples of the same product type and contamination scenario and at the same time interval, suggesting that factors such as frequency, homogeneity, initial levels and types of contamination may affect the natural microflora of the product. Spoilage of aerobically stored product inoculated to simulate plant-contamination was mainly due to presumptive LAB. Growth of these presumptive LAB during vacuum-packaged storage of turkey breast containing lactate-diacetate, and the resulting high levels during aerobic storage may explain the reduced or no significant growth of *L. monocytogenes* during aerobic storage of 25- or 50-day-old product, respectively (Fig. 2). It has been shown that changing the storage environment from microaerophilic or anaerobic to aerobic resulted in faster growth of LAB, as well as in replacement of some members of this bacterial group by others (Samelis et al., 2000). Under the retail/home-contamination scenario, significant increases in counts of presumptive LAB were observed after 9 or 12 and 6-12 days of aerobic storage in product with and without lactate-diacetate, respectively.

Colonies growing on RBC agar were almost exclusively yeasts. Initial levels of yeasts were 0.2 and -0.2 log cfu/cm^2 in turkey breast with and without lactate-diacetate, respectively, and overall, no significant ($P \geqslant 0.05$) increases were observed during vacuum-packaged storage of product inoculated to simulate plant-contamination. Under the same contamination scenario and during aerobic storage, significant growth of yeasts, when observed, was encountered after 6 or 9 days. Growth of yeasts during aerobic storage was also observed under the retail/home-contamination scenario, with significant increases encountered after 6–12 and 6 or 9 days of storage in product with and without lactate-diacetate, respectively. The potentially important contribution of yeasts to spoilage of processed poultry products during refrigerated aerobic storage, as reported by others (Ismail et al., 2000), can be also demonstrated in the present study, even in the presence of antimicrobials. Specifically, in turkey breast with lactate-diacetate that was sliced and inoculated with *L. monocytogenes* after 50 days of storage in vacuum packages, populations of yeasts (log cfu/cm^2) increased from 0.2 on day-0 to 5.6 on day-12 of aerobic storage at 7 °C.

3.4. Chemical and physical properties

Differences between pH values of samples suspended in MRD and distilled water were small (0.01–0.12 units) and pH changes during storage appeared to follow similar trends (data not shown). Sodium diacetate has been known to act as an acidifier when added to the formulation of RTE meats, while salts of lactic acid do not affect the product pH and have been used as pH control agents (Shelef, 1994; Bedie et al., 2001). The initial pH of turkey breast with and without lactate-diacetate was not considerably different in this study, with the pH values of samples suspended in MRD being 6.18 ± 0.02 and 6.26 ± 0.10, respectively. The pH of unsliced turkey breast (both with and without lactate-diacetate), as well as of sliced (inoculated) product containing lactate-diacetate did not change noticeably during storage in vacuum packages (4 °C). However, the pH of sliced product without antimicrobials decreased to 5.76 ± 0.12 after 50 days of vacuum-packaged storage, reflecting microbial growth, most likely that of *L. monocytogenes* and presumptive LAB. Although growth of presumptive LAB was also observed during storage in vacuum packages of product with lactate-diacetate, the more pronounced decrease of pH in product without antimicrobials might have been either due to the prolific growth of *L. monocytogenes*, or due to the predominance of different LAB species in the two products. Consequently, the pH values of samples without antimicrobials inoculated to simulate plant-contamination were generally lower ($P < 0.05$) during subsequent aerobic storage (7 °C) compared to samples with lactate-diacetate or to samples, both with and without antimicrobials, inoculated to simulate retail/home-contamination. In product with lactate-diacetate inoculated to simulate plant-contamination and in product without antimicrobials inoculated to simulate retail/home-contamination, pH decreases ($P < 0.05$), when observed, were encountered after 9 or 12 and 6 days of aerobic storage, respectively. The pH of product containing lactate-diacetate and inoculated to reflect retail/home-contamination did not change significantly during aerobic storage.

The initial a_w of turkey breast was 0.969 ± 0.002 and 0.974 ± 0.002 for samples with and without lactate-diacetate, respectively. The presence of potassium or sodium salts of lactic acid in the formulation of RTE meats has been shown to reduce their a_w (Bedie et al., 2001; Stekelenburg, 2003; Barmpalia et al., 2005). However, in the present study, the a_w of turkey breast with lactate-diacetate was only slightly lower than that of product without antimicrobials. The a_w values of turkey breast, either sliced or unsliced, did not change significantly during refrigerated vacuum-packaged storage and were similar ($P \geqslant 0.05$) in product with and without lactate-diacetate (data not shown).

4. Conclusions

The results demonstrated that the behavior of *L. monocytogenes* in uncured turkey breast during simulated home storage (aerobic storage at 7 °C) depended on contamination scenario and presence of antimicrobials in

the formulation of the product. Plant-contamination (contamination soon after processing) of product without antimicrobials and retail/home-contamination of product containing potassium lactate–sodium diacetate were determined to be the worst and best case scenario, respectively, relative to pathogen levels encountered during subsequent aerobic storage of the product at 7 °C. *L. monocytogenes* populations were lower in product containing potassium lactate–sodium diacetate compared to product without antimicrobials, under both contamination scenarios. Moreover, the results of this study indicated that avoidance of extended shelf life and home storage may reduce considerably the risk of consumer exposure to high levels of the pathogen. The information provided here should be useful in risk assessments and in establishing safety-oriented date labeling for refrigerated RTE meat and poultry products.

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