Conference for Food Protection
2012 Issue Form

Internal Number: 048
Issue: 2012 I-024

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

All information above the line is for conference use only.

Title:
Food Code Date Marking Provision(s) For Raw, Live In-shell SHELLSTOCK

Issue you would like the Conference to consider:
The 2009 FDA Food Code contains no clear guidance (or exception) regarding date marking of raw, live, in-shell MOLLUSCAN SHELLFISH (i.e., SHELLSTOCK) in a FOOD ESTABLISHMENT when the FOOD is served to the CONSUMER in a raw (i.e., not heated treated) form.
This issue submission seeks clarification from the Conference as to date marking of raw, live, in-shell SHELLSTOCK, received and cold held longer than 24 hours in a FOOD ESTABLISHMENT and served to the CONSUMER in a raw (non-heat treated) form.

Public Health Significance:
Per the 2009 FDA Food Code Section 1-201.10 Statement of Application and Listing of Terms, raw, live in-shell SHELLSTOCK served to the CONSUMER without cooking meets the definition of a commercially processed Ready-To-Eat (RTE) Potentially Hazardous [Time/Temperature Control for Safety Food] FOOD (PHF/TCS FOOD) which was previously harvested and subsequently PACKAGED by a FOOD PROCESSING PLANT before being received by a FOOD ESTABLISHMENT.
During the 2004 Conference for Food Protection (CFP) Biennial Meeting, the subject of Food Code date marking for RTE PHF/TCS FOOD was re-evaluated to focus the provision on "Very High" and "High Risk" foods while simultaneously exempting certain categories of FOOD from the date marking provision. The September 2003 document referenced by CFP, Quantitative Assessment of Relative Risk to Public Health from Foodborne *Listeria monocytogenes* Among Selected Categories of Ready-To-Eat Foods, concluded raw seafood to be categorized as "Risk Designation Low" along with other FOOD such as preserved fish products. This designation suggests date marking of raw seafood (including raw, live in-shell SHELLSTOCK) would not be necessary, however neither the 2005 nor the 2009 Food Codes specifically exempt raw, live in-shell SHELLSTOCK from date marking [Section 3-501.17(F)(1-7) Ready-to-Eat, Potentially Hazardous Food (Time/Temperature Control for Safety Food) Date Marking] and no elaborative explanation is offered in Annex 3, 3-501.17 (pages 414-419) regarding raw, live in-shell SHELLSTOCK.
The only guidance in the Food Code is located in Annex 3, 3-201.15 Molluscan Shellfish (pages 374-375) which specifically identifies *Listeria monocytogenes* (and others) as a

pathogen of concern at harvest, a position that is elaborated on in recently published research (Moustafa A. et. al *Listeria spp.* in the coastal environment of the Aqaba Gulf; Suez Gulf and the Red Sea. Epidemiol. Infect. 2006; 134; 752-757) (Colburn KG et. al. *Listeria monocytogenes* in California coast estuarine environment. Applied Environ Microbiol 1990; 56; 2007-2011).

Regarding FOOD excluded from date marking, the 2009 FDA Food Code currently lists only the following commercially produced RTE PHF/TCS FOOD categories: deli salads prepared and packaged in a FOOD PROCESSING PLANT; hard and semi-soft cheeses; cultured dairy products; preserved fish products (with exceptions); shelf stable dry fermented sausages not labeled "Keep Refrigerated"; and shelf stable salt-cured products not labeled "Keep Refrigerated".

Once received by a FOOD ESTABLISHMENT, raw live in-shell SHELLSTOCK are typically cold held longer than 24 hours due to the quantity received. And while the Food Code does not specify the number of days raw, live in-shell SHELLSTOCK can be cold held, Annex 3 estimates a shelf-life up to fourteen (14) days [Section 3-203.12 Shellstock, Maintaining Identification; page 382]. This presents a serious potential challenge to REGULATORY AUTHORITIES that adopt and enforce date marking as recommended in the Food Code since date marking for commercially processed RTE PHF/TCS FOOD limits shelf-life to seven (7) days [Section 3-501.17 Ready-to-Eat, Potentially Hazardous Food (Time/Temperature Control for Safety Food) Date Marking; and Section 3-501.18 Ready-to-Eat, Potentially Hazardous Food (Time/Temperature Control for Safety Food) Disposition].

SHELLSTOCK served in a raw, live in-shell form to the CONSUMER are currently subject to a CONSUMER ADVISORY [Section 3-603.11 Consumption of Animal Foods that are Raw, Undercooked or Not Otherwise processed to Eliminate Pathogens; pages 97-98] and have been identified by FDA as a potential source of pathogen contamination, including *Listeria monocytogenes* [Annex 3; Section 3.201.15 Molluscan Shellfish; page 375].

Further, raw, live in-shell SHELLSTOCK can be harvested, transported and delivered to the FOOD ESTABLISHMENT at temperatures above 41° F [Section 3-202.11 Temperature; page 54] which can encourage the growth of pathogens such as *Listeria monocytogenes*.

Further, SHELLSTOCK are PACKAGED and shipped in netted bags or other non-reusable shipping containers, none of which are air-tight. Some of the non-reusable containers are opened at receiving to allow the FOOD ESTABLISHMENT to verify the condition and temperature of the raw, live in-shell SHELLSTOCK and the porous nature of the shipped non-reusable bags/containers does not discourage or prevent possible further contamination of the SHELLSTOCK under refrigerated storage in the FOOD ESTABLISHMENT.

In the FOOD ESTABLISHMENT, raw, live in-shell SHELLSTOCK are frequently removed from their original shipping container(s) to be: (1) displayed on ice; or (2) held in refrigerated drawers, cold-rails, walk-in-coolers or reach-in-coolers. These refrigerated environments are subject to splash, dust, condensation drips and other filth that may be contaminated with pathogens, including *Listeria monocytogenes*. These refrigeration units can also simultaneously hold other raw animal FOODS and/or other RTE PHF/TCS FOODS. And these refrigeration units can be subject to temperature variation above 41° F as documented in FDA Report on the Occurrence of Foodborne Illness Risk Factors in Selected Institutional Foodservice, Restaurant and Retail Food Store Facility Types (2009) (see attached).

Recommended Solution: The Conference recommends...:

...the language of the 2009 FDA Food Code (as modified by the Supplement issued in 2011) be changed to clearly reflect that date marking provisions apply to raw, live in-shell SHELLSTOCK served to CONSUMERS upon request without cooking or other treatment. *(new language is in underline format; language to be deleted in strike-thru format)*

3-501.17(B) Ready-to-Eat, Potentially Hazardous Food (Time/Temperature Control for Safety Food), Date Marking.

(B) Except as specified in ¶¶ (D)-(F) of this section, refrigerated, READY-TO-EAT, POTENTIALLY HAZARDOUS FOOD (TIME/TEMPERATURE CONTROL FOR SAFETY FOOD) prepared and PACKAGED by a FOOD PROCESSING PLANT shall be clearly marked, at the time the original container is opened in a FOOD ESTABLISHMENT and if the FOOD is held for more than 24 hours, to indicate the date or day by which the FOOD shall be consumed on the PREMISES, sold, or discarded, based on the temperature and time combinations specified in ¶ (A) of this section and:[PF]

(1) The day the original container is opened in the FOOD ESTABLISHMENT shall be counted as Day 1;[Pf] ~~and~~

(a) Except for containers of raw, live in-shell SHELLSTOCK, Day 1 shall be the date or day the SHELLSTOCK are receiving in the FOOD ESTABLISHMENT if the SHELLSTOCK will be served upon CONSUMER request in a raw, RTE PHF/TCS form; [Pf] and

(2) The day or date marking by the FOOD ESTABLISHMENT may not exceed a manufacturer's use-by date if the manufacturer determined the use-by date based on FOOD safety.[Pf]

Submitter Information:

Name: Mark S. Ohlmann, CP-FS
Organization: N/A
Address: 4318 Juniper Forest Place
City/State/Zip: Louisville, KY 40245
Telephone: (502) 552-2204 Fax: (502) 384-2071
E-mail: msohlmann@insightbb.com

Attachments:

- "Listeria monocytogenes Risk Assessment"
- "FDA Report on the Occurrence of Foodborne Illness Risk Factors"
- "Listeria spp. in the coastal environment of the Aqaba Gulf, Suez Gulf and.."
- "Listeria Species in a California Coast Estuarine Environment"

It is the policy of the Conference for Food Protection to not accept Issues that would endorse a brand name or a commercial proprietary process.

Listeria monocytogenes Risk Assessment: Executive Summary

FDA/Center for Food Safety and Applied Nutrition
USDA/Food Safety and Inspection Service
September 2003

Background

The U.S. Department of Health and Human Service, Food and Drug Administration's Center for Food Safety and Applied Nutrition (DHHS/FDA/CFSAN) conducted this risk assessment in collaboration with the U.S. Department of Agriculture's Food Safety and Inspection Service (USDA/FSIS) and in consultation with the DHHS Centers for Disease Control and Prevention (CDC). The purpose of the assessment is to examine systematically the available scientific data and information to estimate the relative risks of serious illness and death associated with consumption of different types of ready-to-eat (RTE) foods that may be contaminated with *Listeria monocytogenes*. This examination of the current science and the models developed from it are among the tools that food safety regulatory agencies will consider when evaluating the effectiveness of current and future policies, programs, and regulatory practices to minimize the public health impact of this pathogen.

The Healthy People 2010 goals for national health promotion and disease prevention called on federal food safety agencies to reduce foodborne listeriosis by 50% by the end of the year 2005. Preliminary FoodNet data on the incidence of foodborne illnesses for the United States in 2001 indicated that the incidence of infection from *Listeria monocytogenes* decreased between 1996 and 2001 from 0.5 to 0.3 cases per 100,000 people per year. The level then reached a plateau. In order to reduce further the incidence to a level of 0.25 cases per 100,000 people by the end of 2005, it became evident that additional targeted measures were needed. The *Listeria monocytogenes* risk assessment was initiated as an evaluation tool in support of this goal.

Listeria monocytogenes is a bacterium that occurs widely in both agricultural (soil, plants and water) and food processing environments. Ingestion of *Listeria monocytogenes* can cause listeriosis, which can be a life-threatening human illness. In 2000, the CDC reported that of all the foodborne pathogens tracked by CDC, *Listeria monocytogenes* had the second highest case fatality rate (21%) and the highest hospitalization rate (90.5%). Serious illness almost always occurs in people considered to be at higher risk, such as the elderly and those who have a pre-existing illness that reduces the effectiveness of their immune system. Perinatal listeriosis results from foodborne exposure of the pregnant mother leading to *in utero* exposure of the fetus, resulting in fetal infection that leads to fetal death, premature birth, or neonatal illness and death. *Listeria monocytogenes* also causes listerial gastroenteritis, a syndrome typically associated with mild gastrointestinal symptoms in healthy individuals. This risk assessment focuses on the severe public health consequences.

Scope and General Approach

This risk assessment provides analyses and models that (1) estimate the potential level of exposure of three age-based population groups and the total United States population to *Listeria monocytogenes* contaminated foods for 23 food categories and (2) relate this exposure to public health consequences. The food categories consist of foods with a documented history of *Listeria monocytogenes* contamination. The models provide a means of predicting the likelihood that severe illness or death will result from consuming foods contaminated with this pathogen. These

predictions are interpreted and used to estimate the relative risks among the food categories. The foods considered in this risk assessment are ready-to-eat foods that are eaten without being cooked or reheated just prior to consumption. One food, frankfurters, may or may not be reheated prior to consumption and was considered as two separate food categories. The working assumption is that all the cases of listeriosis are attributed to the foods in 23 categories, so that the risk assessment could be 'anchored' to the United States public health statistics. However, it is recognized that additional foods or cross-contamination from raw foods before cooking to other RTE foods may also contribute to additional cases.

The published scientific literature, government food intake surveys, health statistics, epidemiological information, unpublished food product surveys acquired from state and federal public health officials and trade associations, and surveys specifically designed to augment the data available for the risk assessment are the primary sources of data used in this document. Expert advice on scientific assumptions was actively sought from leading scientists from academia, industry, and government. This included two formal reviews of the underlying model structure and assumptions by the United States National Advisory Committee on Microbiological Criteria for Foods. In addition, the risk assessment was initially published in draft form and public comments sought for six months.

While the risk assessment purposely did not look into the pathways for the manufacture of individual foods, the risk assessment model developed can be used to estimate the likely impact of control strategies by changing one or more input parameters and measuring the change in the model outputs. This process, referred to as conducting 'what-if' scenarios, can be used to explore how the components of a complex model interact. Several 'what-if' scenarios are detailed within the risk assessment to evaluate the impact of refrigerator temperature, storage times, and reduction of the number of organisms in foods at before it is sold, and reduction in the contamination levels in foods that support growth.

Results

The relative risk rankings, along with the corresponding risk estimates expressed in terms of both the predicted number of cases per serving and per annum, are provided in Summary Table 1. Both measures are important in understanding and interpreting the risks associated with foodborne listeriosis. The per serving value can be considered the inherent risk associated with the manufacturing, distribution, marketing, and use of the food category, and is reflective of the degree of *Listeria monocytogenes* control achieved. Examples of factors that influence the 'per serving' risk include the frequency of contamination, the extent of that contamination, the ability of the food category to support the growth of *Listeria monocytogenes*, the duration and temperature of refrigerated storage, and the size of the serving. The predicted relative risk per serving can be viewed as the relative risk faced by individual consumers when he/she consume a single serving of the various foods considered in this risk assessment. The 'per serving' risk is typically the value upon which risk management decisions related to a specific product are based.

Summary Table 4. Relative Risk Ranking and Predicted Median Cases of Listeriosis for the Total United States Population on a per Serving and per Annum Basis

Relative Risk	Predicted Median Cases of Listeriosis for 23 Food Categories	
	Per Serving	Per Annum Basis[b]

Ranking	Basis[a]					
	Food	Cases	Food	Cases		
1	**High Risk**	Deli Meats	$7.7x10^{-8}$	**Very High**	Deli Meats	1598.7
2		Frankfurters, not reheated	$6.5x10^{-8}$	**High Risk**	Pasteurized Fluid Milk	90.8
3		Pâté and Meat Spreads	$3.2x10^{-8}$		High Fat and Other Dairy Products	56.4
4		Unpasteurized Fluid Milk	$7.1x10^{-9}$		Frankfurters, not reheated	30.5
5		Smoked Seafood	$6.2x10^{-9}$	**Moderate Risk**	Soft Unripened Cheese	7.7
6		Cooked Ready-to-Eat Crustaceans	$5.1x10^{-9}$		Pâté and Meat Spreads	3.8
7	**Moderate Risk**	High Fat and Other Dairy Products	$2.7x10^{-9}$		Unpasteurized Fluid Milk	3.1
8		Soft Unripened Cheese	$1.8x10^{-9}$		Cooked Ready-to-Eat Crustaceans	2.8
9		Pasteurized Fluid Milk	$1.0x10^{-9}$		Smoked Seafood	1.3
10	**Low Risk**	Fresh Soft Cheese	$1.7x10^{-10}$	**Low Risk**	Fruits	0.9
11		Frankfurters, reheated	$6.3x10^{-11}$		Frankfurters, reheated	0.4
12		Preserved Fish	$2.3x10^{-11}$		Vegetables	0.2
13		Raw Seafood	$2.0x10^{-11}$		Dry/Semi-dry Fermented Sausages	<0.1
14		Fruits	$1.9x10^{-11}$		Fresh Soft Cheese	<0.1
15		Dry/Semi-dry Fermented Sausages	$1.7x10^{-11}$		Semi-Sof t Cheese	<0.1

16		Semi-soft Cheese	$6.5x10^{-12}$		Soft Ripened Cheese	<0.1
17		Soft Ripened Cheese	$5.1x10^{-12}$		Deli-type Salads	<0.1
18		Vegetables	$2.8x10^{-12}$		Raw Seafood	<0.1
19		Deli-type Salads	$5.6x10^{-13}$		Preserved Fish	<0.1
20		Ice Cream and Other Frozen Dairy Products	$4.9x10^{-14}$		Ice Cream and Other Frozen Dairy Products	<0.1
21		Processed Cheese	$4.2x10^{-14}$		Processed Cheese	<0.1
22		Cultured Milk Products	$3.2x10^{-14}$		Cultured Milk Products	<0.1
23		Hard Cheese	$4.5x10^{-15}$		Hard Cheese	<0.1

[a] Food categories were classified as high risk (>5 cases per billion servings), moderate risk (≤5 but ≥1 case per billion servings), and low risk (<1 case per billion servings).
[b] Food categories were classified as very high risk (>100 cases per annum), high risk (>10 to 100 cases per annum), moderate risk (≥1 to 10 cases per annum), and low risk (<1 cases per annum).

The second measure, the 'per annum risk,' is the predicted number of fatal infections per year in the United States for each food category. This value takes into account the number of servings of the food category that are consumed. The predicted per annum risk of serious illnesses for each food category can be thought of as the predicted relative total public health impact for each food category. Since the 'per annum' risk is derived from the 'per serving' risk, there is generally a higher degree of uncertainty associated with the former. The predicted per serving and per annum relative risks are used to develop risk rankings to compare the various food categories. In addition to presenting the 'most likely' relative risk rankings for the different population groups and food categories, the risk assessment provides the inherent variability and the uncertainty associated with these rankings.

Evaluation and Interpretation

The overall interpretation of the risk assessment requires more than just a simple consideration of the relative risk rankings associated with the various food categories. First, the interpretation of the results requires an appreciation of the fact that the values being compared are the median values of distributions that may be highly skewed (i.e., not evenly distributed). The use of median values was selected as being the appropriate method for comparing the overall relative risks among the different food categories. The quantitative results must be considered in relation to the associated variability and uncertainty (i.e., the confidence intervals surrounding the median) and interpreted in the context of both the epidemiologic record and how the food categories are manufactured, marketed, and consumed. A detailed consideration of the

quantitative and qualitative findings for each food category is provided in the risk assessment and its appendices.

A number of methods for objectively grouping the results were evaluated, and are discussed in detail within the risk assessment. One approach is cluster analysis. When performed at the 90% confidence level, this analysis groups the per serving rankings into four clusters and the per annum rankings into five. These clusters are used, in turn, to develop a two-dimensional matrix of per serving vs. per annum rankings of the food categories (Summary Figure 1). In this approach, the 'per serving' clusters are arrayed against the 'per annum' clusters. The matrix is then used to depict five risk designations: Very High, High, Moderate, Low, and Very Low.

The risk characterization combines the exposure and dose-response models to predict the relative risk of illness attributable to each food category. While the risk characterization must be interpreted in light of both the inherent variability and uncertainty associated with the extent of contamination of ready-to-eat foods with *Listeria monocytogenes* and the ability of the microorganism to cause disease, the results provide a means of comparing the relative risks among the different food categories and population groups considered in the assessment and should prove to be a useful tool in focusing control strategies and ultimately improving public health through effective risk management. As described above, cluster analysis techniques are employed as a means of discussing the food categories within a risk analysis framework. The food categories are divided into five overall risk designations (see Summary Figure 1), which are likely to require different approaches to controlling foodborne listeriosis.

	Decreased Risk per Annum ⟶			
	Clusters A and B	**Clusters C and D**	**Cluster E**	
Decreased Risk per Serving ↓	**Cluster 1**	**Very High Risk (Clusters 1-A, 1-B)** Deli Meats Frankfurters (not reheated)	**High Risk (Clusters 1-C, 1-D)** Pâté and Meat Spreads Unpasteurized Fluid Milk Smoked Seafood	**Moderate Risk (Cluster 1-E)** No food categories
	Cluster 2	**High Risk (Clusters 2-A, 2-B)** High Fat and Other Dairy Products Pasteurized Fluid	**Moderate Risk (Clusters 2-C, 2-D)** Cooked RTE Crustaceans	**Moderate Risk (Cluster 2-E)** No food categories

		Milk Soft Unripened Cheese		
	Cluster 3	**Moderate Risk (Clusters 3-A, 3-B)** No food categories	**Moderate Risk (Clusters 3-C, 3-D)** Deli-type Salads Dry/Semi-dry Fermented Sausages Frankfurters (reheated) Fresh Soft Cheese Fruits Semi-soft Cheese Soft Ripened Cheese Vegetables	**Low Risk (Cluster 3-E)** Preserved Fish Raw Seafood
	Cluster 4	**Moderate Risk (Clusters 4-A, 4-B)** No food categories	**Low Risk (Clusters 4-C, 4-D)** No food categories	**Very Low Risk (Cluster 4-E)** Cultured Milk Products Hard Cheese Ice Cream and Other Frozen Dairy Products Processed Cheese

Summary Figure 1. Two-Dimensional Matrix of Food Categories Based on Cluster Analysis of Predicted per Serving and per Annum Relative Rankings

[The matrix was formed by the interception of the four per serving clusters vs. the per annum clusters A and B, C and D, and E. For example, Cluster 3-E (Low Risk) refers to the food categories that are in both Cluster level 3 for the risk per serving and Cluster level E for the risk per annum.]

Risk Designation Very High. This designation includes two food categories, Deli Meats and Frankfurters, Not Reheated. These are food categories that have high predicted relative risk rankings on both a per serving and per annum basis, reflecting the fact that they have relatively high rates of contamination, support the relatively rapid growth of *Listeria monocytogenes* under refrigerated storage, are stored for extended periods, and are consumed extensively. These products have also been directly linked to outbreaks of listeriosis. This risk designation is one that is consistent with the need for immediate attention in relation to the national goal for reducing the incidence of foodborne listeriosis. Likely activities include the development of new control strategies and/or consumer education programs suitable for these products.

Risk Designation High. This designation includes six food categories, High Fat and Other Dairy Products, Pasteurized Fluid Milk, Pâté and Meat Spreads, Soft Unripened Cheeses, Smoked Seafood, and Unpasteurized Fluid Milk. These food categories all have in common the ability to support the growth of *Listeria monocytogenes* during extended refrigerated storage. However, the foods within this risk designation appear to fall into two distinct groups based on their rates of contamination and frequencies of consumption.

- Pâté and Meat Spreads, Smoked Seafood, and Unpasteurized Fluid Milk have relatively high rates of contamination and thus high predicted per serving relative risks. However, these products are generally consumed only occasionally in small quantities and/or are eaten by a relatively small portion of the population, which lowers the per annum risk. All three products have been associated with outbreaks or sporadic cases, at least internationally.

  These foods appear to be priority candidates for new control measures (i.e., Smoked Seafood, Pâté and Meat Spreads) or continued avoidance (i.e., Unpasteurized Fluid Milk).
- High Fat and Other Dairy Products, Pasteurized Fluid Milk, and Soft Unripened Cheeses have low rates of contamination and corresponding relatively low predicted per serving relative risks. However, these products are consumed often by a large percentage of the population, resulting in elevated predicted per annum relative risks. In general, the predicted per annum risk is not matched with an equivalent United States epidemiologic record. However, the low frequency of recontamination of individual servings of these products in combination with their broad consumption makes it likely that these products are primarily associated with sporadic cases and normal case control studies would be unlikely to lead to the identification of an association between these products and cases of listeriosis.

  These products (High Fat and Other Dairy Products, Pasteurized Fluid Milk, and Soft Unripened Cheeses) appear to be priority candidates for advanced epidemiologic and scientific investigations to either confirm the predictions of the risk assessment or identify the factors not captured by the current models that would reduce the predicted relative risk.

Risk Designation Moderate. This risk designation includes nine food categories (Cooked Ready-to-Eat Crustaceans, Deli Salads, Fermented Sausages, Frankfurters-Reheated, Fresh Soft Cheese, Fruits, Semi-soft Cheese, Soft Ripened Cheese, and Vegetables) that encompass a range of contamination rates and consumption profiles. A number of these foods include effective bactericidal treatments in their manufacture or preparation (e.g., Cooked Ready-to-Eat Crustaceans, Frankfurters-Reheated, Semi-soft Cheese) or commonly employ conditions or compounds that inhibit the growth of *Listeria monocytogenes* (e.g., Deli Salads, Dry/Semi-dry Fermented Sausages). The risks associated with these products appear to be primarily associated with product recontamination, which in turn, is dependent on continued, vigilant application of proven control measures.

Risk Designation Low. This risk designation includes two food categories, Preserved Fish and Raw Seafood. Both products have moderate contamination rates but include conditions (e.g., acidification) or consumption characteristics (e.g., short shelf-life) that limit *Listeria monocytogenes* growth and thus limit predicted per serving risks. The products are generally consumed in small quantities by a small portion of the population on an infrequent basis, which results in low predicted per annum relative risks. Exposure data for these products are limited so

there is substantial uncertainty in the findings. However, the current results predict that these products, when manufactured consistent with current good manufacturing practices, are not likely to be a major source of foodborne listeriosis.

Risk Designation Very Low. This risk designation includes four food categories, Cultured Milk Products, Hard Cheese, Ice Cream and Other Frozen Dairy Products, and Processed Cheese. These products all have in common the characteristics of being subjected to a bactericidal treatment, having very low contamination rates, and possessing an inherent characteristic that either inactivates *Listeria monocytogenes* (e.g., Cultured Milk Products, Hard Cheese) or prevents its growth (e.g., Ice Cream and Other Frozen Dairy Products, Processed Cheese). This results in a very low predicted per serving relative risks. The predicted per annum relative risks are also low despite the fact that these products are among the more commonly consumed ready-to-eat products considered by the risk assessment. The results of the risk assessment predict that unless there was a gross error in their manufacture, these products are highly unlikely to be a significant source of foodborne listeriosis.

Conclusions

The following conclusions are provided as an integration of the results derived from the models, the evaluation of the variability and uncertainty underlying the results, and the impact that the various qualitative factors identified in the hazard identification, exposure assessment, and hazard characterization have on the interpretation of the risk assessment.

- The risk assessment reinforces past epidemiological conclusions that foodborne listeriosis is a moderately rare although severe disease. United States consumers are exposed to low to moderate levels of *Listeria monocytogenes* on a regular basis.
- The risk assessment supports the findings of epidemiological investigations of both sporadic illness and outbreaks of listeriosis that certain foods are more likely to be vehicles for *Listeria monocytogenes*.
- Three dose-response models were developed that relate the exposure to different levels of *Listeria monocytogenes* in three age-based subpopulations [i.e., perinatal (fetuses and newborns), elderly, and intermediate-age] with the predicted number of fatalities. These models were used to describe the relationship between levels of *Listeria monocytogenes* ingested and the incidence of listeriosis. The dose of *Listeria monocytogenes* necessary to cause listeriosis depends greatly upon the immune status of the individual.
    1. Susceptible subpopulations (such as the elderly and perinatal) are more likely to contract listeriosis than the general population.
    2. Within the intermediate-age subpopulation group, almost all cases of listeriosis are associated with specific subpopulation groups with increased susceptibility (e.g., individuals with chronic illnesses, individuals taking immunosuppressive medication).
    3. The strong association of foodborne listeriosis with specific population groups suggests that strategies targeted to these susceptible population groups, i.e., perinatal (pregnant women), elderly, and susceptible individuals within the intermediate-age group, would result in the greatest reduction in the public health impact of this pathogen.
- The dose-response models developed for this risk assessment considered, for the first time, the range of virulence observed among different isolates of *Listeria monocytogenes*.

The dose-response curves suggest that the relative risk of contracting listeriosis from low dose exposures could be less than previously estimated.>

- The exposure models and the accompanying 'what-if' scenarios identify five broad factors that affect consumer exposure to *Listeria monocytogenes* at the time of food consumption.
    1. Amounts and frequency of consumption of a ready-to-eat food
    2. Frequency and levels of *Listeria monocytogenes* in a ready-to-eat food
    3. Potential of the food to support growth of *Listeria monocytogenes* during refrigerated storage
    4. Refrigerated storage temperature
    5. Duration of refrigerated storage before consumption

Any of these factors can affect potential exposure to *Listeria monocytogenes* from a food category. These factors are 'additive' in the sense that foods where multiple factors favor high levels of *Listeria monocytogenes* at the time of consumption are typically more likely to be riskier than foods where a single factor is high. These factors also suggest several broad control strategies that could reduce the risk of foodborne listeriosis such as reformulation of products to reduce their ability to support the growth of *Listeria monocytogenes* or encouraging consumers to keep refrigerator temperatures at or below 40 °F and reduce refrigerated storage times. For example, the 'what-if' scenarios using Deli Meats predicts that consumer education and other strategies aimed at maintaining home refrigerator temperatures at 40 °F could substantially reduce the risks associated with this food category. Combining this with pre-retail treatments that decrease the contamination levels in Deli Meats would be expected to reduce the risk even further.

This risk assessment significantly advances our ability to describe our current state of knowledge about this important foodborne pathogen, while simultaneously providing a framework for integrating and evaluating the impact of new scientific knowledge on public health enhancement.

FDA Report on the Occurrence of Foodborne Illness Risk Factors in Selected Institutional Foodservice, Restaurant, and Retail Food Store Facility Types (2009)

EXECUTIVE SUMMARY

In 2008, the U.S. Food and Drug Administration's (FDA) National Retail Food Team conducted the third phase of a three-phase,10-year study to measure the occurrence of practices and behaviors commonly identified by the Centers for Disease Control and Prevention (CDC) as contributing factors in foodborne illness outbreaks. Specifically, the study called for conducting data collection inspections of various types of foodservice and retail food establishments at five-year intervals to observe and document practices and behaviors that relate to the following CDC contributing factor categories associated with foodborne illness outbreaks within foodservice and retail food establishments, herein referred to as foodborne illness risk factors (risk factors):

- Food from Unsafe Sources
- Poor Personal Hygiene
- Inadequate Cooking
- Improper Holding/Time and Temperature
- Contaminated Equipment/Protection from Contamination

This 2009 report is the third report in a series and presents findings based on data collected in 2008. The first report in the study was issued in August 2000 and presented the findings from the first data collection effort in 1998. A second report was issued in 2004 and presented data collected in 2003. FDA intends to publish a report in 2010 that compares the results from the three data collection periods and examines what trends, if any, were observed.

The 2000 and 2004 reports called attention to the need for greater active managerial control of foodborne illness risk factors. They suggested that more innovative and effective strategies to improve food safety practices in retail and foodservice establishments were needed. The reports highlighted operational areas most in need of improvement including employee handwashing, cold holding of potentially hazardous foods (time/temperature control for safety foods), date marking of ready-to-eat foods, and cleaning and sanitizing of food contact surfaces.

In each phase of the study, FDA Regional Retail Food Specialists collected data during site visits of over 800 establishments representing nine distinct facility types. Direct observations, supplemented with information gained from discussions with management and food employees, were used to document the establishments' compliance status for 42 individual data items based on provisions in the *1997 FDA Food Code*. In each establishment, the compliance status for each data item was recorded in terms of IN Compliance, Out of Compliance, Not Observed (meaning the behavior or practice was applicable but not observed during the visit), or Not Applicable (meaning the behavior or practice did not apply to the establishment).

For each of the nine facility types, the percentage of observations recorded as Out of Compliance is presented for each risk factor and for the individual data items related to those risk factors most in need of priority attention. The percent Out of Compliance value for each risk factor was calculated by dividing the total number of Out of Compliance observations of data items in the

risk factor by the total number of observations (IN compliance and Out of Compliance) of data items in the risk factor. The percent Out of Compliance values for individual data items were calculated by dividing the total number of Out of Compliance observations for the individual data item by the total number of observations (IN and Out of Compliance) for the data item.

The data presented in this 2009 report indicate that some of the same risk factors and data items identified as problem areas in the 2000 and 2004 Reports remain in need of priority attention. This indicates that industry and regulatory efforts to promote active managerial control of these risk factors must be enhanced. The Out of Compliance percentages remained high for data items related to the following risk factors:

- Improper Holding/Time and Temperature
- Poor Personal Hygiene
- Contaminated Equipment/Protection from Contamination

For the improper holding/time and temperature risk factor, the high percent Out of Compliance values were most commonly associated with improper cold holding of potentially hazardous food (PHF)/time-temperature control for safety (TCS) food and inadequate date marking of refrigerated, ready-to-eat PHF/TCS Food.

Within the poor personal hygiene risk factor, the proper, adequate handwashing data item had the highest percent Out of Compliance value for every facility type. Percent Out of Compliance values for proper, adequate handwashing ranged from approximately 18% for meat departments to approximately 76% for full service restaurants.

Of the data items related to the contaminated equipment/protection from contamination risk factor, improper cleaning and sanitizing of food-contact surfaces before use was the item most commonly observed to be Out of Compliance in eight out of the nine facility types. Percent Out of Compliance values for this data item ranged from 18% in seafood departments to 64% in full service restaurants.

As in the 2004 Report, this 2009 report includes a comparison between the data collected from food establishments that had a Certified Food Protection Manager (CFPM) from a program recognized by the Conference for Food Protection and those that did not. The results of the study indicate that the presence of a Certified Food Protection Manager is positively correlated to the overall IN Compliance percentages in certain facility types, especially in delis, full service restaurants, seafood departments, and produce departments. Poor Personal Hygiene, Improper Holding/Time and Temperature, and Contaminated Equipment/Protection from Contamination appear to be the risk factors for which the presence of a certified manager had the most positive correlation.

The 2003 and 2008 data collection efforts included several supplemental data items that were not included in the 1998 data collection. While original 42 data items in the study remained the same from 1998 to 2008, the supplemental data items addressed changes made to the *FDA Food Code* since 1997. These items related to the cooking temperature for pork, the minimum hot holding temperatures, employee health, juice, eggs, and highly susceptible populations. Data gathered for the supplemental data items suggest that reducing the minimum hot holding temperature for PHF/TCS foods from 140ºF (60ºC) to 135ºF (57ºC) and reducing the minimum cooking temperature for pork from 155ºF (68ºC) to 145ºF (63ºC) had minimal effect on the industry's control of these risk factors.

Results from the 2008 data collection indicate that the recommendations made to foodservice and retail food operators and regulators in the 2000 and 2004 Reports need to be

reemphasized. Foodservice and retail food store operators must ensure that their management systems are designed to achieve active managerial control over the risk factors. Likewise, regulators must ensure that their inspection, education, and enforcement efforts are geared toward the control of the risk factors commonly found to be Out of Compliance.

Epidemiol. Infect. (2006), **134**, 752–757. © 2005 Cambridge University Press
doi:10.1017/S0950268805005601 Printed in the United Kingdom

Listeria spp. in the coastal environment of the Aqaba Gulf, Suez Gulf and the Red Sea

MOUSTAFA A. EL-SHENAWY[1]* AND MOHAMED A. EL-SHENAWY[2]

[1] *National Research Centre, Dokki, Cairo 12311, Egypt*
[2] *National Institute of Oceanography and Fisheries, El-Anfoshi, Alexandria, Egypt*

(*Accepted 22 September 2005, first published online 22 December 2005*)

SUMMARY

Listeria monocytogenes is an important pathogen which causes an infection called listeriosis. Because of the high mortality rate (~30%) associated with listeriosis, and the widespread nature of the organism, it is a major concern for food and water microbiologists since it has been isolated from various types of foods, including seafood, as well as from the aqueous environment. To investigate the prevalence of this pathogen in the Aqaba Gulf (12 sites), Suez Gulf (14 sites) and Red Sea (14 sites), 200 water samples (collected during five sampling cruises in 2004), 40 fresh fish samples and 15 shellfish samples were analysed using the enrichment procedure and selective agar medium. All water samples were also examined for the presence *Listeria innocua* which was the most common of the *Listeria* spp. isolated, followed by *L. monocytogenes*, with a low incidence of the other species. During the whole year, the percentage of *Listeria* spp. and *L. monocytogenes* in 200 water samples was 20·5% (41 samples) and 13% (26 samples) respectively. In fresh fish (40 samples) it was 37% (15 samples) and 17·3% (7 samples) and in shellfish (15 samples) 53% (8 samples) and 33% (5 samples) respectively. In water samples, there was an association between the faecal contamination parameters and the presence of the pathogen; however, water salinity, temperature, dissolved oxygen and pH did not influence the occurrence of this bacterium. These results may help in the water-quality evaluation of the coastal environments of these regions.

INTRODUCTION

Listeria monocytogenes has been recognized as a human pathogen since 1929 [1] causing an infection called listeriosis which can be manifested through several different syndromes causing invasive illness. It can cause abortion during pregnancy, human meningitis, infection during the perinatal period, granulomatosis infantiseptica, sepsis, diarrhoea, pyelitis and 'flu-like' symptoms. The mortality rate of listeriosis is ~30% [2].

* Author for correspondence: Professor M. A. El-Shenawy, Department of Microbiology, National Research Center, Dokki, Cairo 12311, Egypt.
(Email: m_elshenawy@hotmail.com)

In the early 1980s scientists recognized *Listeria* as a foodborne pathogen as human listerosis resulted from consuming food contaminated with this pathogen such as milk and dairy products, meat, poultry, vegetables, salads and seafood [3]. Moreover, *L. monocytogenes* has a saprophytic life and occurs widely in nature [4]. A variety of animals including domestic farm animals can carry the bacterium [5], and it can survive for long periods in a plant–soil environment [6]. *Listeria* spp. were isolated from the Mediterranean coast of Egypt, more specifically from the Eastern Harbour of Alexandria [7], while in the United States the bacterium was isolated from the California coast estuarine environment [6]. Scientists have proposed that the pathogen can survive for

a long time in a marine environment as it is a salt-tolerant organism [8–10].

In Egypt, the coastal water of Red Sea including the Suez Gulf and the Aqaba Gulf, may be contaminated by domestic and/or industrial sewage/wastes. Thus, the risk of the presence of such a pathogen in these contaminated areas is to be expected. *Listeria* spp. have been recovered from a variety of seafood [11–13]; in Egypt, they have been isolated from fresh fish [14] as well as from shellfish collected from the Eastern Harbour of Alexandria [7].

This work addresses the incidence of *Listeria* spp. as well as the faecal pollution bacteria indicators in the coastal marine water of the Aqaba Gulf, Suez Gulf and Red Sea. Testing of seafood, collected from the local markets along the investigated areas, for the presence of the pathogen was another goal of the study.

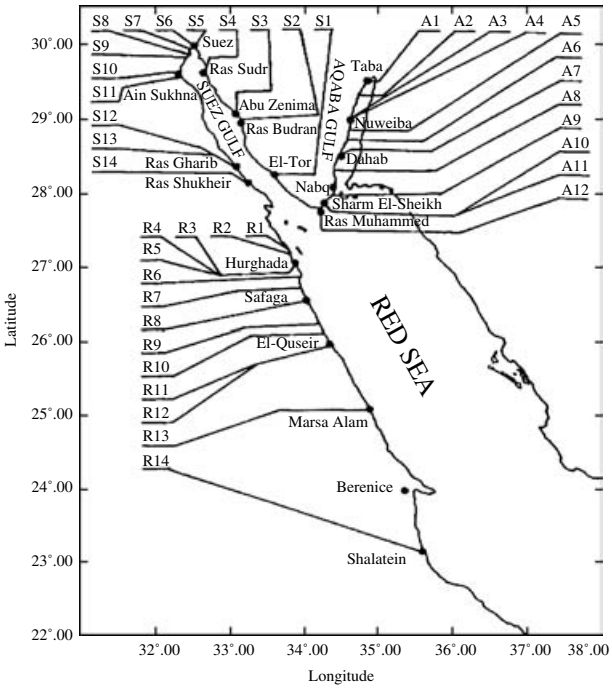

Fig. 1. Map showing names and codes of sampling locations at Aqaba Gulf (A), Suez Gulf (S) and Red Sea (R).

MATERIALS AND METHODS

Sampling sites

Coastal water samples were collected in five sampling cruises (bi-monthly intervals) during February to October 2004. The sampling sites along the Aqaba Gulf, Suez Gulf and Red Sea are shown in Figure 1. Water was sampled using 1-litre screw-cap bottles, with two sample bottles being taken at the same time/place from each site.

At each sampling cruise, one fresh fish sample, was purchased from the fish markets of the cities of Nuweiba, Sharm El-Sheikh, Suez, Ras Gharib, Hurghada, Safaga, El-Quseir and Shalatein; one shellfish sample was also purchased, but only from Nuwebia, Suez and Safaga.

All water samples were analysed immediately using an on-site mobile microbiological laboratory. At each sampling site, hydrographical parameters of the water samples including temperature (°C), salinity (‰), dissolved oxygen (mg/l and pH) were measured using CTD (YSI 6000, Yellow Springs, OH, USA). Fish and shellfish samples were collected in plastic bags and kept in the refrigerator of the mobile laboratory and were analysed within 6–12 h.

Bacteriological analysis

Water samples were examined for the presence of faecal pollution indicators of bacteria including total coliforms, *E. coli* and faecal streptococci using the membrane filtration technique (Gelman 0·45 μm membranes) as described by ISO 9308/1 [15], and ISO 7899/2 [16]. For detection of total coliforms, the membranes were fixed onto m-Endo-LES agar and incubated at 37 °C for 24 h; for detection of *E. coli*, m-FC agar was used followed by incubation at 44·5 °C for 24 h; however for faecal streptococci, m-Enterococcus agar was used and incubated at 37 °C for 48 h. The biochemical tests used for confirmation of the characteristic colonies as well as calculation of the final bacterial counts, per 100 ml of seawater, were done after the above-mentioned ISO examinations.

For detection of *Listeria* spp., the enrichment procedure based on the US Food and Drug Administration (FDA) technique [17] was used. One litre of each water sample was filtered. More than one membrane was used for each sample when needed. Then, the filter membrane(s) of each sample were scrubbed and manually crushed, using a sterile sharp glass rod, in a 500-ml volume sterile beaker containing 100 ml of *Listeria* enrichment broth base (LEB) (Oxoid CM 882 broth, Oxoid, Basingstoke, Hampshire, UK) supplemented with *Listeria* selective supplement (Oxoid SR 141). Membrane suspensions were transferred to sterilized conical flasks and kept at refrigerator temperature (4–8 °C) for 4 weeks.

Shellfish, after being scrubbed and rinsed with tap water, were opened aseptically and the flesh was collected. The flesh, or fish samples, were then blended in a sterile grinder to achieve homogenated slurries. Twenty-five grams of each representative slurry was directly suspended in 225 ml of LEB, and kept at refrigerator temperature (4–8 °C) for 4 weeks. The refrigerated enrichment cultures were then streaked onto Oxford formula selective agar medium (CM 856) plus *Listeria* selective supplement (SR 140) as described by Curtis et al. [18]. Plates were incubated at 35 °C for 48 h. Up to four colonies that were presumptively positive *Listeria*, with a black halo and a sunken centre, from each suspected positive plate were picked and purified onto trypticase soy agar (TSA) plates. For identification of the isolates a positive control of *L. monocytogenes* strain V7 (milk isolate) serotype 1 (obtained from the Department of Food Science, University of Wisconsin, Madison, WI, USA) served as a control in this work. This isolate was recovered by all media used in this study. Purified suspected isolates were viewed by the oblique light technique of Henry as described by the IDF [19]. Smears of suspected grey-blue colonies were Gram-stained and examined microscopically after streaking onto TSA plates and incubated at 35 °C for 18–24 h. Cultures displaying the correct morphology were tested for catalase, then stabbed into motility test medium (Difco, Detroit, MI, USA) and incubated at 25 °C to check for umbrella-shaped growth. Cultures that proved to be motile were tested for haemolysin using tryptose agar with 5% sheep blood. The isolates were streaked onto TSA slants, incubated at 35 °C for 18–24 h for further characterization to species using the criteria described by McLauchlin [2].

RESULTS AND DISCUSSION

Figure 2 shows the faecal pollution indicators represented as c.f.u./100 ml of the water samples examined during whole year, as well as the prevalence of *Listeria* spp. in the three studied areas.

In the Aqaba Gulf area *Listeria* spp. were detected in 10 out of 60 (17%) samples investigated during the whole year. Five of these contaminated samples (50%) were found to harbour *L. monocytogenes*. In the Suez Gulf area, 16 out of 70 (23%) samples investigated, proved to be contaminated by *Listeria* spp. Twelve (75%) of these contaminated samples were found to harbour *L. monocytogenes*. Along the coastal area of the Red Sea, only 15 out of 70 (21%) samples investigated were contaminated by *Listeria* spp., where nine (60%) of them harboured *L. monocytogenes*. These results indicated that Suez Gulf recorded the highest percentage for the presence of the bacterium. This may be due to the drainage of wastes and/or untreated sewage into the Gulf.

Generally, as illustrated in Table 1, *Listeria* spp. were detected in 21% ($n=41$) of the total examined water samples collected from all the investigated areas during the whole year ($n=200$). This percentage is lower than those reported at the Eastern harbour of Alexandria, where 9 out of 11 (82%), surface water samples were found to harbour *Listeria* spp. These results are similar to those reported in the United States where the percentage amounted to 33% of the examined marine waters collected from the California coast estuarine environment. Of these contaminated water samples ($n=41$), 63% ($n=26$) were found to harbour *L. monocytogenes*, however, *L. innocua* was the most predominant of the *Listeria* spp. since it was found in 80% ($n=33$) of the contaminated samples. At the same time a small percentage (5%) of other *Listeria* spp. was also detected ($n=2$).

In general, in the results obtained, *L. innocua* was more prevalent than *L. monocytogenes*, suggesting that it might be a very common organism in the coastal environment. Such an observation should, however, be made with care, since only a few colonies of the pathogen were picked up from the selective plates for identification. In this regard Seeliger [20] reported that *L. innocua* is a good indicator for *L. monocytogenes*, thus, when looking for sources of *Listeria*, the presence of both of these species is equally significant.

The present results indicated that there was an association between the faecal pollution indicators and the presence of the pathogen. As seen in Figure 2, the pathogen was detected in sites with high bacterial counts of the three faecal pollution indicators and never isolated from any site with low counts for these parameters. This association was to be expected since the bacterium is widely distributed in sewage [21] and the numbers of *Listeria* that are contributed to the environment by sewage and sewage sludge may be higher than those of *Salmonella* [22].

It should be noted that such an association between the presence of the pathogen and the faecal contamination indicators should not be considered as a general trend, since many factors/relations may interfere, e.g. distribution in water, sediments and biofilms,

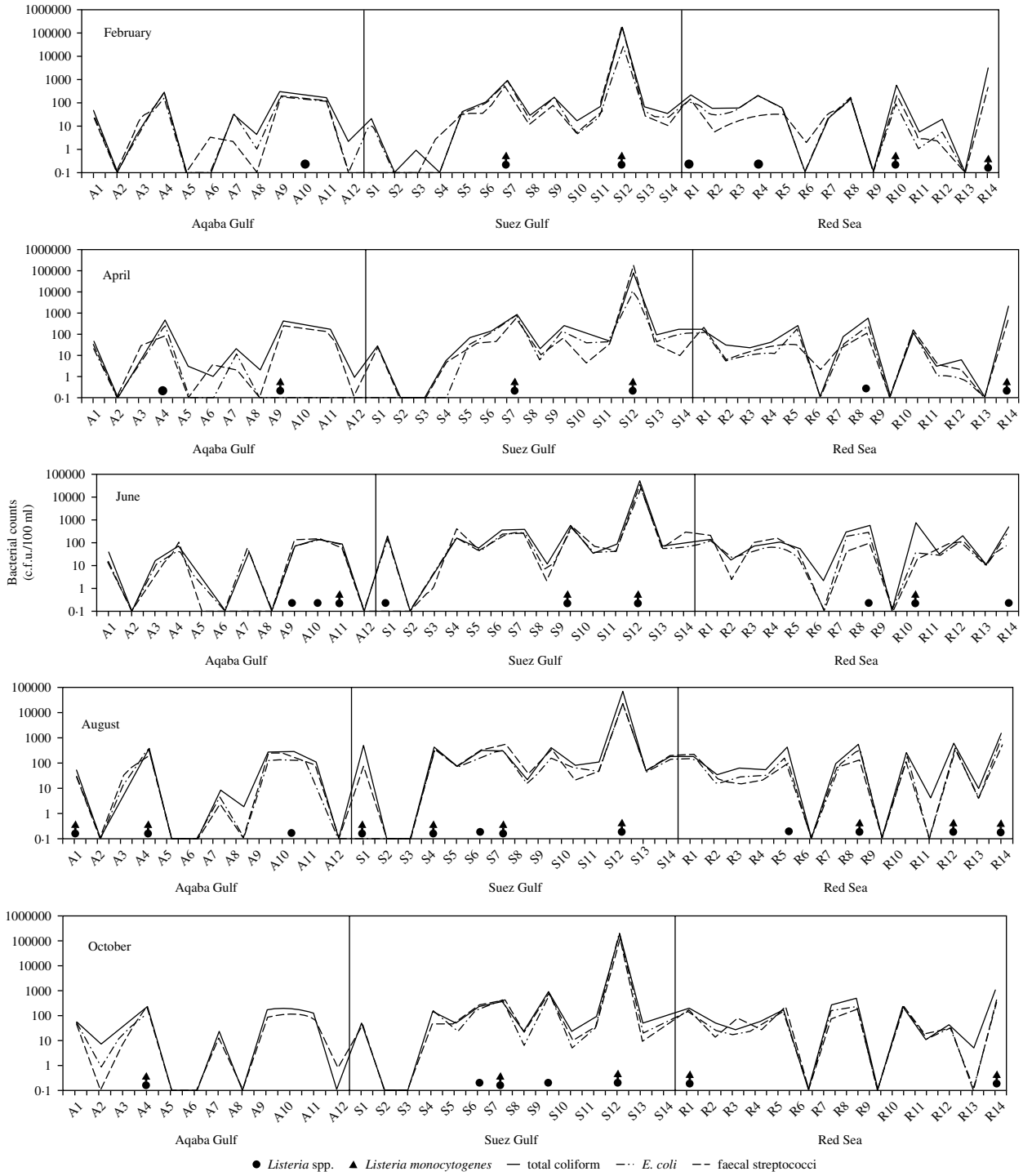

Fig. 2. Distribution of *Listeria* spp. and counts of faecal bacteria in coastal waters of Aqaba Gulf (A), Suez Gulf (S), and the Red Sea (R) during 2004.

seasonal variations, total bacterial load, relationship with indicators, etc. At least, our data give preliminary information on the behaviour of *Listeria* spp. in this coastal environment in an attempt to understand the epidemiology of this pathogen. At the same time, the recorded hydrographical parameters indicated that seasonal variation affected water temperature which ranged between 17·7 and 28·5 °C. A range of 39·2–42·6‰ for salinity was, however, recorded; the pH ranged from 7·8 to 8·5 and the dissolved oxygen

Table 1. *Number and percentage of water, fish and shellfish samples positive for* Listeria *spp.*

Sample	No. of examined samples	No. of positive samples				
		L. spp.	*L.m.*	*L.i.*	*L.m.* + *L.i.*	Other spp.
Water	200	41 (21%)	6 (3%)	13 (7%)	20 (10%)	2 (1%)
Fish	40	15 (38%)	3 (8%)	8 (20%)	4 (10%)	—
Shellfish	15	8 (53%)	1 (6%)	3 (20%)	4 (27%)	—

L. spp., *Listeria species*; *L.m.*, *Listeria monocytogenes*; *L.i.*, *Listeria innocua*; *L.m.* + *L.i.*, both species together.

Table 2. Listeria *spp. in fish and shellfish samples*

Location	Type	No. of samples	No. of samples positive for	
			Listeria spp.	*Listeria monocytogenes*
Fish				
Nuweiba	*Sigan*, *Hemiramphus*	5	2	1
Sharm El-Sheikh	*Lethrinus*, Shrimp	5	2	1
Suez	*Lethrinus*, *Sepia Hemiramphus*	5	3	2
Ras Garib	*Lethrinus*, *Mullus*	5	1	1
Hurghada	*Sigan*, squids, *Mullus*	5	1	—
Safaga	Shrimp, Sea bream	5	1	—
El-Quseir	*Lethrinus*, *Sigan*	5	2	1
Shalatein	*Lethrinus*, Sea bream	5	3	1
Total		40	15	7
Shellfish				
Nuweiba	*Lithophaga*, *Tridacna*	5	2	1
Suez	*Lambis*, caretshell Oyster	5	3	2
Safaga	*Lithophaga*, *Donax*	5	3	2
Total		15	8	5

between 6·3 and 9·5 mg/l. This variation in these hydrographical parameters, during the whole year, did not appear to affect the distribution of *Listeria* spp. and/or the bacteria of faecal pollution indicators in the investigated sites.

The incidence of *Listeria* spp. in the examined fish and shellfish samples is summarized in Table 2. As can be seen, *L. innocua* and *L. monocytogenes* were the only two species found in the investigated seafood samples. The incidence of *Listeria* spp. in the positive samples in relation to fish/shellfish type and the location where it was purchased is presented in Table 2.

In fish 38% ($n=15$) of the total examined samples ($n=40$) were found to be contaminated by *Listeria* spp. while 17·0% ($n=7$) were contaminated with *L. monocytogenes*. As observed in the coastal water samples, *L. innocua* was the most prevalent species since it was isolated from 30% ($n=12$) of the total samples examined (Table 1). These results are in accordance with reports by other authors [6] where the incidence of such a pathogen in fresh fish varied from very low to up to 50%, while a percentage ranging from 14·8 to 72·4% for the presence of *Listeria* spp. was also reported.

The highest frequency of *Listeria* spp. was recovered from shellfish where 53% ($n=8$) of the total examined samples ($n=15$) were contaminated with *Listeria* spp. and 33% ($n=5$) harboured *L. monocytogenes*. As was found in fish, *L. innocua* was the

most prevalent since it was isolated from 47% ($n=7$) of the total samples examined (Table 1). These results are a little higher than those reported by Colburn et al. [6] (they found a range from 12 to 44·4% for the presence of *Listeria* spp. and from 4·0 to 12·1% for *L. monocytogenes*). In general, the numbers of the samples examined, the pumping rate by shellfish which provokes the accumulation of microorganisms, the ability of *Listeria* spp. to survive in marine waters and the degree to which *Listeria* spp. are diluted are all different factors that may affect the uptake and retention of *Listeria* spp. by the shellfish; and consequently, the numbers of positive samples that might be affected. Moreover, the percentages of the pathogen in fish/shellfish could not be linked to the coastal environment only, this may also be linked to the market environment, although most fish markets in these areas are very close to the seashore.

In conclusion, faecal pollution bacteria as well as *Listeria* spp. were detected in some sites along the investigated areas. The polluted sites were located either in front of populated cities such as the Suez Gulf area or in front of the industrial/tourism activities in the other investigated areas. Consequently, the discharge of domestic raw/partially treated sewage onto the polluted sites should be taken into consideration. Our results may draw attention to the need to implement better hygiene and epidemiological practices in these areas. In order to avoid listerosis infection, fish and shellfish must be well-cooked before consumption.

DECLARATION OF INTEREST

None.

REFERENCES

1. **Gray ML, Killinger AH.** *Listeria monocytogenes* and listeric infection. Bacteriol Rev 1966; **30**: 309–382.
2. **McLauchlin J.** *Listeria monocytogenes*, recent advances in the taxonomy and epidemiology of listeriosis in humans. J Appl Bacteriol 1987; **63**: 1–11.
3. **El-Shenawy MA, Marth EH.** *Listeria monocytogenes* and foods: history, characteristics, implication, isolation methods and control: a review. Egypt J Dairy Sci 1989; **17**: 1–18.
4. **Prentice GA.** Living with *Listeria*. J Soc Dairy Technol 1989; **42**: 55–58.
5. **Gitler M.** 1985 Listeriosis in farm animals in Great Britain. In Collins CH, Grange JM (eds). Isolation and identification of micro-organisms of medical and veterinary importance: 191–200. London: Academic Press.
6. **Colburn KG, Kaysner CA, Abeyta C, Wekell C, Wekell MM.** *Listeria monocytogenes* in California coast estuarine environment. Appl Environ Microbiol 1990; **56**: 2007–2011.
7. **El-Shenawy MA, El-Shenawy MA.** *Listeria* species in some aquatic environments in Alexandria, Egypt. Int J Environ Health Res 1996; **6**: 131–140.
8. **Conner DE, Brackett RE, Bauchat LR.** Effect of temperature, sodium chloride, and pH on growth of *Listeria monocytogenes* in cabbage juice. Appl Environ Microbiol 1986; **52**: 59–63.
9. **Shahamat M, Seamon A, Woodbine M.** Survival of *Listeria monocytogenes* in high salt concentrations. Zentralbl Bakteriol Parasitenkd Infektionskr Hyg Abt 1 Orig Reihe A 1980; **46**: 506–511.
10. **Elmanseer N, Bakhrouf A.** Survival study of *Listeria monocytogenes* in seawater. Rapp Comm Int Mer Médit 2004; **37**: 273.
11. **Weagent SD, Sado PN, Colburn KG, et al.** The incidence of *Listeria* species in frozen seafood products. J Food Prot 1988; **51**: 655–657.
12. **Laciar AL, de Centorbi ONP.** *Listeria* species in seafood in San Luis; Argentina. Food Microbiol 2002; **19**: 645–651.
13. **Karunasager I, Karunasager I.** *Listeria* in tropical fish and fishery products. Int J Food Microbiol 2002; **62**: 177–181.
14. **El-Shenawy MA, El-Shenawy MA.** Incidence of *Listeria monocytogenes* in seafood enhanced by prolonged enrichment. J Med Inst, Alex Univ ARE 1995; **16**: 32–40.
15. **ISO (International Organization for Standardization).** No. 9308/1, 1990. Water quality detection and enumeration of coliform organisms, thermotolerant coliform organisms and presumptive *Escherichia coli* – Part 1: Membrane filtration method. Geneva, Switzerland.
16. **ISO (International Organization for Standardization).** No. 7899/2, 1984. Water quality detection and enumeration of streptococci – Part 2: Methods by membrane filtration. Geneva, Switzerland.
17. **Lovett J, Francis DW, Hunt JM.** *Listeria monocytogenes* in raw milk: detection, incidence and pathogenicity. J Food Prot 1987; **50**, 188–192.
18. **Curtis GDW, Mitchell RG, King AF, Emma J.** A selective differential medium for the isolation of *Listeria monocytogenes*. Lett Appl Microbiol 1989; **8**, 95–98.
19. **IDF (International Dairy Federation).** Detection of *Listeria monocytogenes*. IDF standard 1990; **143**: 1–8.
20. **Seeliger HP.** Listeriosis – history and actual developments. Infection 1988; **16** (Suppl 2): 580–584.
21. **Al-Ghazali WR, Al-Azawi SK.** Detection and enumeration of *Listeria monocytogenes* in sewage treatment plant in Iraq. J Appl Bacteriol 1986; **60**: 251–254.
22. **Watkins J, Sleath KP.** Isolation and enumeration of *Listeria monocytogenes* from sewage, sludge and river. J Appl Bacteriol 1981; **50**: 1–9.

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 1990, p. 2007–2011 Vol. 56, No. 7
0099-2240/90/072007-05$02.00/0

Listeria Species in a California Coast Estuarine Environment

KAREN G. COLBURN, CHARLES A. KAYSNER, CARLOS ABEYTA, JR., AND MARLEEN M. WEKELL*

Seafood Products Research Center, U.S. Food and Drug Administration, 22201 23rd Drive S.E., P.O. Box 3012, Bothell, Washington 98041-3012

Received 18 December 1989/Accepted 13 April 1990

***Listeria* species and *L. monocytogenes* were found in 81 and 62%, respectively, of fresh or low-salinity waters (37 samples) in tributaries draining into Humboldt-Arcata Bay, Calif., during a winter (January-February) sampling period. The incidence of *Listeria* species and *L. monocytogenes* in sediment (46 samples) from the same sites where water was sampled was 30.4 and 17.4%, respectively. One of three bay water samples contained *Listeria* species (including *L. monocytogenes*), while of 35 samples of oysters examined, only 1 was found positive for *Listeria* species (*L. innocua*). A given species or *L. monocytogenes* serogroup appeared to predominate in fresh water when domesticated animals (cows, horses) were nearby, whereas greater variety with no species predominance was observed in areas with no direct animal influence.**

Listeria monocytogenes has been implicated in recent foodborne outbreaks (7, 14–16, 20) which have focused attention on this organism and its modes of introduction into foods. A variety of animals including domestic farm animals can carry *Listeria* species in both infectious and latent states and are therefore considered potential vectors of this organism (3, 6, 8, 9, 12). It has been suggested (24, 26) that *Listeria* species are saprophytic and capable of surviving for long periods in a plant-soil environment. This factor may also play a role in transmission of this organism to foods.

Listeria species are present in aqueous environments such as river waters and sewage sludge (22) and most recently have been recovered from a variety of seafood products (23). Although *L. monocytogenes* can tolerate salt (4, 21), it is not known whether it can reach marine waters via freshwater tributaries or whether it is capable of prolonged survival in marine environments. Therefore, whether its presence in seafoods is due to environmental or postprocessing contamination or a combination of these and other factors is presently unknown.

This study was conducted to determine the incidence of *Listeria* species in freshwater tributaries draining into Humboldt-Arcata Bay, Calif. This estuary supports an active molluscan shellfishery and is impacted by humans and domesticated and wild animals.

MATERIALS AND METHODS

Samples and sites. Sediment, freshwater, saltwater, and oyster samples were collected over 13 consecutive days during January–February 1988. Specific sites sampled included those along various tributaries and portions of Arcata Bay (Fig. 1). Fresh water was sampled at sites 1 to 9, sediment samples were collected from sites 1 to 5 and 8, saltwater sampling locations were at sites 10 to 12, and oysters were from sites 13 to 17. Water and sediment samples were maintained at ambient temperature; oysters were kept on ice after sampling. All samples were analyzed within 6 h of collection by using an on-site mobile microbiological laboratory. At each sampling, water temperature was taken with a mercury thermometer and salinity was measured with either a salinometer (Beckman Instruments, Inc., Fullerton, Calif.) or a refractometer (Atago Co. Ltd., Tokyo, Japan). The visual observation of domesticated farm animals near the sampling site was noted. In each case in which the animals were present, they were within 200 m of the sampling site.

Sample collection. (i) Water. Water was sampled by three methods as follows. (A) Surface water samples were collected with sterile 4-liter screw-cap plastic bottles (Nalgene Labware Div., Nalge/Sybron Corp., Rochester, N.Y.) (sites 1 to 5 and 8, Fig. 1). (B) Sterile Moore swabs (sterile gauze pads) (2, 13, 18, 25) were suspended on a string for 7 to 8 days in situ approximately 1 m below the water surface at seven freshwater and three saltwater sampling stations (sites 1 and 4 to 12, Fig. 1). After 7 to 8 days, the swab was removed from the water, placed in a sterile plastic bag (Whirlpak; Nasco, Fort Attenson, Wis.), transported at ambient temperatures to the laboratory, and analyzed within 2 h. (C) A sterile Moore swab was placed within 2 h of collection in a 4-liter surface water sample in a plastic collection bottle and held in the laboratory at 22°C for 18 to 24 h. The Moore swab was then removed from the collection container and placed directly in 225 ml of nutrient broth (NB) (Difco Laboratories, Detroit, Mich.). For analysis of salt water (salinity, >30‰), only the examination of Moore swabs (method B) was used.

(ii) Sediment. Approximately 200 g of surface layer (2 to 5 cm) sediment was collected with sterile plastic scoops. Samples were placed in sterile Whirlpak bags. Sediment consistency was noted by visual observation (Table 1).

(iii) Oysters. Pacific oysters (*Crassostrea gigas*) in plastic mesh bags were attached to floats and suspended 0.5 to 2 m below the surface of Arcata Bay and not in contact with the bottom for 2 weeks before sampling. Bags contained 14 to 15 oysters. One bag was removed from each station at each sampling, and 12 oysters from each bag were analyzed.

Bacteriological analysis. (i) Water. A 1- to 2-liter volume was filtered through a 0.45-μm-pore-size 142-mm membrane filter (Millipore Corp., Bedford, Mass.). The filter was blended for 5 to 10 s in 225 ml of NB. All Moore swabs including those having an extended exposure in situ and those suspended in water samples in the laboratory were placed in 225 ml of NB.

(ii) Sediment. Sediments in plastic bags were mixed, and 25 g from each bag was added to 225 ml of NB.

(iii) Oysters. Oysters were scrubbed, rinsed with tap water, shucked, and blended for 90 s (1). Portions (25 g) of

* Corresponding author.

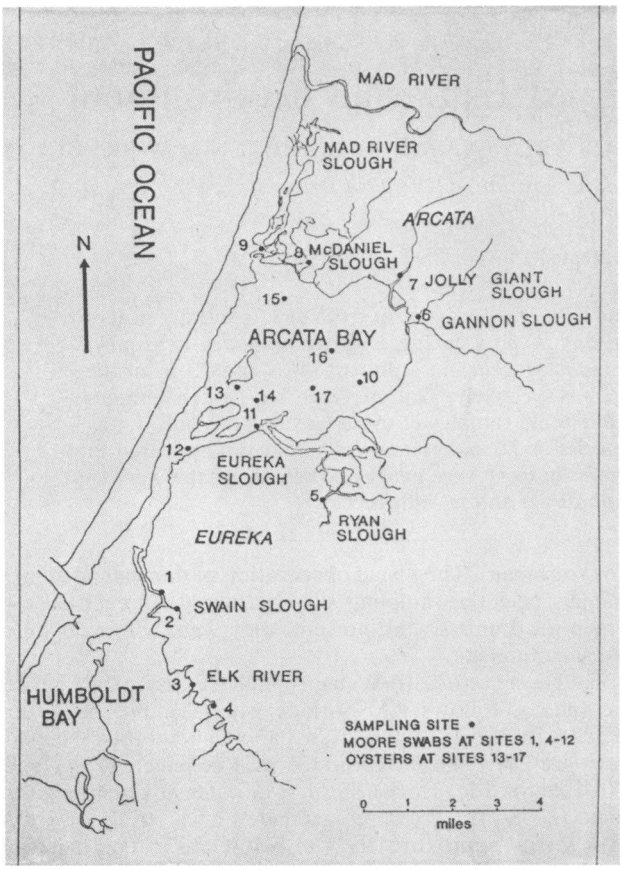

FIG. 1. Map of Humboldt-Arcata Bay area sampling sites. Fresh water, sites 1 to 9; sediment, sites 1 to 5 and 8; seawater, sites 10 to 12; and oyster, sites 13 to 17.

oyster homogenate were added to 225 ml of listeria enrichment broth (17) and to NB.

Enrichment and isolation procedures. The NB was incubated at 4°C (9, 22, 24) and after 7 and 28 days, 1 ml was transferred to 9 ml of thiocyanate-nalidixic acid nutrient broth (TN) (22) and incubated for 18 to 24 h at 35°C. Both undiluted and diluted (1:10, in 0.5% KOH) TN broth was streaked onto modified McBride agar (17) and incubated for 48 h at 35°C. The listeria enrichment broth was incubated and streaked as directed previously (17). A positive control consisting of *L. monocytogenes* V-7 serotype 1a:1 was inoculated into sterile NB and listeria enrichment broth, incubated, and recovered by all media used in this study.

Biochemical tests. The modified McBride agar plates were viewed by the oblique lighting technique of Henry (10, 17). Suspect gray-blue colonies, randomly selected, were stabbed into 7% sheep blood agar (Blood Agar Base no. 2; Oxoid Ltd., London, England) to detect the hemolysin reaction and into motility test medium (Difco) and held at 22°C for further confirmatory tests. Isolates were checked for purity by streaking onto tryptic soy agar containing 0.6% yeast extract (TSA-YE) (Difco) and incubated for 18 to 24 h at 35°C. Isolates from TSA-YE that were catalase positive and had characteristic tumbling motility in a wet mount were further characterized by the procedure described by Lovett (17).

L. monocytogenes isolates were serotyped (17) with Difco antisera for groups 1 and 4 and then further serotyped with more specific antisera provided by R. Bennett (Food and Drug Administration, Washington, D.C.). Final confirmation was conducted by colony hybridization (5, 11) with a ^{32}P-labeled oligonucleotide probe for the *Listeria* beta-hemolysin gene (5) supplied by F. M. Harrell (Food and Drug Administration, Minneapolis, Minn.).

RESULTS AND DISCUSSION

Fresh water. *Listeria* species were detected in 81% (n = 37) of freshwater samples. This is similar to the report of

TABLE 1. Distribution of *Listeria* species in freshwater and sediment samples

Source[a]	Sample type	No. of samples[b]	Temp (°C)	Salinity (‰)	Sediment composition	No. of samples positive for:					
						Listeria species	*L. monocytogenes* serotypes (1a:1/4b:6/4)	*L. innocua*	*L. seeligeri*	*L. welshimeri*	*L. ivanovii*
Elk River											
Site 1	Water	8*	8–12	5–17		7	0/4/0	3	1	4	0
	Sediment	3			Silt	0	0/0/0	0	0	0	0
Site 2+	Water	1	9.5	0.8		1	0/0/0	0	0	1	0
	Sediment	1			Silt	0	0/0/0	0	0	0	0
Site 3+	Water	2	7.5–11	0–0.6		2	2/2/0	0	0	1	0
	Sediment	4			Sand	3	1/0/0	0	1	1	0
Site 4+	Water	5*	8–10	0–0.7		4	0/4/0	1	0	1	0
	Sediment	4			Sand	3	0/1/0	0	0	2	0
Ryan Slough (site 5+)	Water	7*	6–11	0.2–1.2		6	0/4/1	0	2	2	0
	Sediment	22			Silt	6	1/5/0	1	1	1	0
McDaniel Slough (site 8)	Water	11*	8.5–12	0–0.7		10	5/3/1	2	8	6	1
	Sediment	12			Soil	2	0/0/0	0	2	0	0
Mad River, McDaniel Slough, delta+ (six sites in 1-mile [1.6-km] area)	Sediment	15	11–15	0–0.9	Peat	3	0/0/0	0	0	3	0

[a] +, Animals observed near sampling site.
[b] *, Total includes a Moore swab placed at this location for 7 to 8 days in addition to surface water samples collected.

Watkins and Sleath (22) of recovering *Listeria* species in all river waters (n = 7) sampled in the United Kingdom. *L. monocytogenes* (1a:1, 4b:6, or 4) was isolated from 62% of all water samples and was the most predominant of *Listeria* species (Table 1). A wide variety of *Listeria* species and, frequently, more than one species were isolated from each location (Table 1).

Two of the three techniques used here for analyzing freshwater samples, analysis of filters and analysis of Moore swabs incubated in the sample collection bottle in the laboratory, were effective for recovery of *Listeria* species at each sampling site. No *Listeria* species were recovered from Moore swabs suspended in situ for 7 to 8 days at each of seven freshwater sampling stations. Why organisms attached to Moore swabs in the laboratory but not to gauze suspended in situ is unknown. Perhaps attachment to gauze is affected by temperature, incubation time, salinity, or other conditions existing as a function of enclosure within the plastic sample container.

Sediment. *Listeria* spp. were recovered from 30.4% of 46 sediment samples collected at the same locations as the surface water samples (sites 1 to 5 and 8). The predominant species recovered, *L. monocytogenes*, was isolated from 17.4% of the 46 samples. Besides the sites sampled above, an additional 15 sediment samples having the consistency of peat were collected from the edges of a slow-flowing drainage system through grazing areas where sheep, cows, ducks, and geese were present. These samples were collected at six distinct sampling sites approximately 0.25 to 0.5 mile (0.4 to 0.8 km) apart in a delta region between McDaniel Slough and Mad River (Fig. 1). *L. welshimeri* was the only *Listeria* species isolated and was detected in 3 of the 15 samples (20%).

Despite the lower overall incidence of *Listeria* species in sediment compared with fresh water, the rate was similar to the 20.9% incidence of *Listeria* species recovered by Weis and Seeliger (24) from sediment in the south of the Federal Republic of Germany.

The proximity of domesticated animals to a sample site appeared to affect the incidence and predominant species recovered. Sediment samples from Elk River (sites 3 and 4) and Ryan Slough (site 5), which had domesticated animals nearby, had a higher incidence of *Listeria* species (75, 75, and 27.3%, respectively) than did sediment from those sites where animals were absent, such as from Elk River (site 1) (no *Listeria* species recovered) or McDaniel Slough, (site 8) (16.7%).

Distribution in fresh water and sediment. Why *Listeria* species are more prevalent in fresh water than in sediment was not determined but is probably due to a number of factors. Differences in species composition and levels of indigenous competing bacteria between different sample types and other conditions noted at the sample site such as the influence of animals, urbanization, changes in salinity due to tidal activity in the area, or sediment type (26) may also affect the apparent overall distribution and recovery of *Listeria* species in water compared with sediment at a given site.

These data indicate that the incidence of *Listeria* species remains high throughout the freshwater tributaries entering Humboldt-Arcata Bay. A given species or serogroup predominated in fresh water when domesticated animals were in close proximity to the sample site. For example, *L. monocytogenes* (4b:6) was predominant in water at Elk River (site 4). At site 3, *L. monocytogenes* serotypes 1a:1 and 4b:6 were predominant (cows were observed at both sites 3 and 4). *L. monocytogenes* (4b:6) was the main species isolated from waters of Ryan Slough, (site 5) (Table 1), where horses were observed. Both horses and cows can be sources of *Listeria* species (3, 8, 9, 12). The variety of *Listeria* species isolated from water appeared to be greater and no one particular species or serogroup predominated at sites without observable direct domesticated animal and/or human influence. This was illustrated at the Elk River (site 1) and McDaniel Slough (site 8) (Table 1), sites impacted by runoff from the urban area of Arcata, Calif. Direct animal influence was not observed at either site.

Slight variations in salinity due to tidal action did not appear to affect the distribution of *Listeria* species in this water system. Tidal influence was greatest for Elk River (site 1) (5 to 17‰ salinity); four *Listeria* species were recovered from 87% of samples from this location (Table 1). This is similar to data obtained at a site of negligible salinity (McDaniel Slough, site 8) where 90% of water samples were positive for *Listeria* species (five species isolated).

These data indicate that there was a consistent input of *Listeria* species from these freshwater tributaries draining into Humboldt-Arcata Bay. *Listeria* species could also be introduced to the bay via other sources. For example, *L. monocytogenes* (4b:6) and *L. innocua* were isolated from a water sample from an urban drain in Eureka, Calif., which emptied directly into Humboldt Bay. In addition, the influence of a large local seagull population observed here and the presence of other marine birds can also be a consistent source of *Listeria* species to the marine environment (6).

Bay water. Although Moore swabs suspended in situ were not effective for recovering *Listeria* species from fresh water, *Listeria* species were isolated from one (site 11) of three Moore swabs placed in situ in marine waters (sites 10 to 12). *Listeria* species recovered from this swab sample included *L. monocytogenes* 1a:1 and 1a:2, *L. innocua*, and *L. welshimeri*. The presence of *Listeria* species in marine water may indicate a recent contamination since a study (A. T. Fuad, S. D. Weagant, M. M. Wekell, and J. Liston, Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, Q243, p. 370) has shown that *L. monocytogenes* levels decrease when low levels are inoculated into seawater. Effects of dilution by the large volumes of seawater in the marine environment may also result in lower levels of *Listeria* species in marine compared with fresh waters.

Oysters. *L. innocua* was isolated from 1 of 35 oyster samples analyzed from five different sites in Arcata Bay (Fig. 1) and was the only *Listeria* species found in oysters. This is the lowest incidence rate (2.8%) by sample type observed in this study (Fig. 2). The ability of *Listeria* species to survive in marine waters, the degree to which *Listeria* species are diluted, and the pumping rate by oysters are all factors that could affect the uptake and retention of *Listeria* species by oysters.

All *L. monocytogenes* isolated in this study gave a positive reaction with the oligonucleotide probe for the hemolysin gene. No other *Listeria* species isolated in this study reacted with the probe.

Conclusion. *Listeria* species were consistently recovered over a 13-day sampling period during the winter from freshwater tributaries draining into Humboldt-Arcata Bay. These tributaries, which are impacted by domestic farm animals, can contribute *Listeria* species to the Humboldt-Arcata Bay system. The incidence of *Listeria* species in sediments (30.4%) was much lower compared with the incidence in fresh water (81%). This difference could be due to a variety of reasons such as different levels of available nutrients,

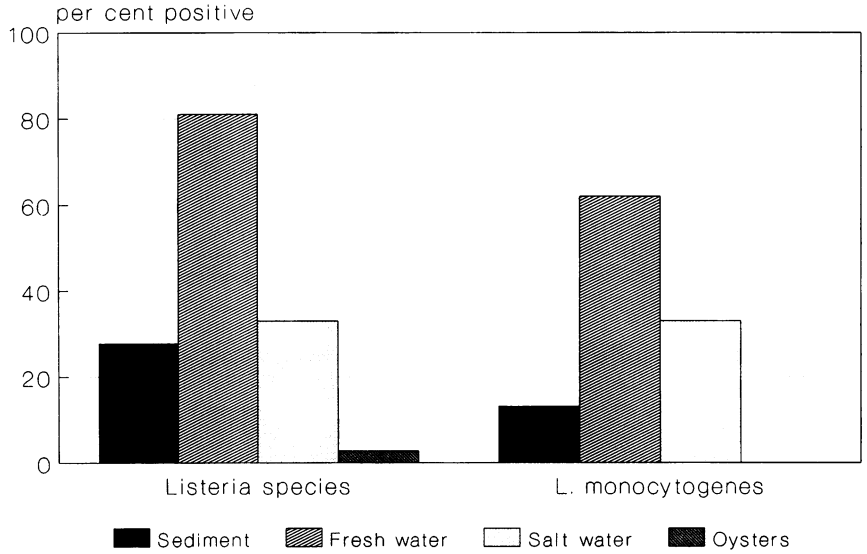

FIG. 2. Incidence of total *Listeria* species and *L. monocytogenes* by sample type.

presence of toxic compounds, and predation by other organisms (19). It is also possible that the lower incidence of recovered *Listeria* species could reflect an initiation of a viable but nonculturable state response by *Listeria* species to these various conditions. Although this survival strategy has not yet been demonstrated for *Listeria* species, it has been shown for a number of other microorganisms and is reviewed by Roszak and Colwell (19). Although the apparent incidence of *Listeria* species is lower in marine waters (33%) compared with fresh waters and was lowest in oysters (2.8%), *Listeria* species were detected throughout the watershed and therefore can be introduced to oysters raised there. These data suggest that the incidence of *Listeria* species is low in oysters held in this estuary during the winter months and most probably represents recent contamination from terrestrial sources.

ACKNOWLEDGMENTS

We thank Coast Oyster Co., Eureka, Calif., for supplying oyster samples, David Alton, Loyman Skaggs, John Mussleman, and Jack Gaines (Food and Drug Administration) for sample collection of marine samples, and Nancy Hill for typing the manuscript.

LITERATURE CITED

1. **American Public Health Association.** 1970. Recommended procedures for the examination of sea water and shellfish, 4th ed. American Public Health Association, New York.
2. **Barrett, T. J., P. A. Blake, G. K. Morris, N. D. Puhr, H. B. Bradford, and J. G. Wells.** 1980. Use of Moore swabs for isolating *Vibrio cholerae* from sewage. J. Clin. Microbiol. **11**:385–388.
3. **Blenden, D. C., and F. T. Szatalowicz.** 1967. Ecological aspects of listeriosis. J. Am. Vet. Med. Assoc. **151**:1761–1766.
4. **Conner, D. E., R. E. Brackett, and L. R. Bauchat.** 1986. Effect of temperature, sodium chloride, and pH on growth of *Listeria monocytogenes* in cabbage juice. Appl. Environ. Microbiol. **52**:59–63.
5. **Datta, A. R., B. N. Wentz, and W. E. Hill.** 1987. Detection of hemolytic *Listeria monocytogenes* by using DNA colony hybridization. Appl. Environ. Microbiol. **53**:2256–2259.
6. **Fenlon, D. R.** 1985. Wild birds and silage as reservoirs of *Listeria* in the agriculturable environment. J. Appl. Bacteriol. **59**:537–543.
7. **Fleming, D. W., S. L. Cochi, K. L. MacDonald, J. Brandum, P. S. Hayes, B. D. Plikaytis, M. B. Holmes, A. Audurier, C. V. Broome, and A. L. Reingold.** 1985. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. N. Engl. J. Med. **312**:404–407.
8. **Gitler, M.** 1985. Listeriosis in farm animals in Great Britain, p. 191–200. *In* C. H. Collins and J. M. Grange (ed.), Isolation and identification of micro-organisms of medical and veterinary importance. Academic Press, Inc. (London), Ltd., London.
9. **Gray, M. L., and A. H. Killinger.** 1966. *Listeria monocytogenes* and listeric infections. Bacteriol. Rev. **30**:309–382.
10. **Henry, B. S.** 1933. Dissociation of the genus *Brucella*. J. Infect. Dis. **52**:374–402.
11. **Hill, W. E.** 1987. Detection of pathogenic bacteria by DNA colony hybridization. FDA bacteriological analytical manual, 6th ed., Ch. 26. Association of Official Analytical Chemists, Arlington, Va.
12. **Hird, D. W.** 1987. Review of evidence for zoonotic listeriosis. J. Food Prot. **50**:429–433.
13. **Kaysner, C. A., C. Abeyta, Jr., M. M. Wekell, A. DePaola, Jr., R. F. Stott, and J. M. Leitch.** 1987. Incidence of *Vibrio cholerae* from estuaries of the United States west coast. Appl. Environ. Microbiol. **53**:1344–1348.
14. **Kvenberg, J. E.** 1988. Outbreaks of listeriosis/*Listeria*-contaminated foods. Microbiol. Sci. **5**:355–358.
15. **Lennon, D., B. Lewis, C. Mantell, D. Becroft, B. Dove, K. Farmer, S. Tonkin, N. Yeates, R. Stamp, and K. Mickleson.** 1984. Epidemic perinatal listeriosis. Pediatr. Infect. Dis. **3**: 30–34.
16. **Linnan, M. J., et al.** 1985 Epidemic listeriosis associated with Mexican-style cheese. N. Engl. J. Med. **319**:823–828.
17. **Lovett, J.** 1987. *Listeria* isolation. FDA bacteriological analytical manual, 6th ed., Ch. 29. Association of Official Analytical Chemists, Arlington, Va.
18. **Moore, B., E. L. Perry, and S. T. Chard.** 1952. A survey by the sewage swab method of latent enteric infection in an urban area. J. Hyg. **50**:137–156.
19. **Roszak, D. B., and R. R. Colwell.** 1987. Survival strategies of bacteria in the natural environment. Microbiol. Rev. **51**:365–379.
20. **Schlech, W. F., P. M. Lavigne, R. A. Bortolussi, A. C. Allen, E. V. Haldane, A. J. Wort, A. W. Hightower, S. E. Johnson, S. H. King, E. S. Nicholls, and C. V. Broome.** 1983. Epidemic listeriosis—evidence for transmission by food. N. Engl. J. Med. **308**:203–206.
21. **Shahamat, M., A. Seamon, and M. Woodbine.** 1980. Survival of *Listeria monocytogenes* in high salt concentrations. Zentralbl.

Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **46**:506–511. Cited by M. P. Doyle. 1988. Effect of environmental and processing concentrations on *Listeria monocytogenes*. J. Food Technol. **42**:169–171.

22. **Watkins, J., and K. P. Sleath.** 1981. Isolation and enumeration of *Listeria monocytogenes* from sewage, sewage sludge and river water. J. Appl. Bacteriol. **50**:1–9.
23. **Weagant, S. D., P. N. Sado, K. G. Colburn, J. D. Torkelson, F. A. Stanley, M. H. Krane, S. C. Shields, and C. F. Thayer.** 1988. The incidence of *Listeria* species in frozen seafood products. J. Food Prot. **51**:655–657.
24. **Weis, J., and H. P. R. Seeliger.** 1975. Incidence of *Listeria monocytogenes* in nature. Appl. Microbiol. **30**:29–32.
25. **Wells, J. G., G. K. Morris, and P. S. Brachmon.** 1971. New method of isolating *Salmonella* from milk. Appl. Microbiol. **21**:235–259.
26. **Welshimer, H. J.** 1960. Survival of *Listeria monocytogenes* in soil. J. Bacteriol. **80**:316–320.