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Listeria Species in a California Coast Estuarine Environment

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***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

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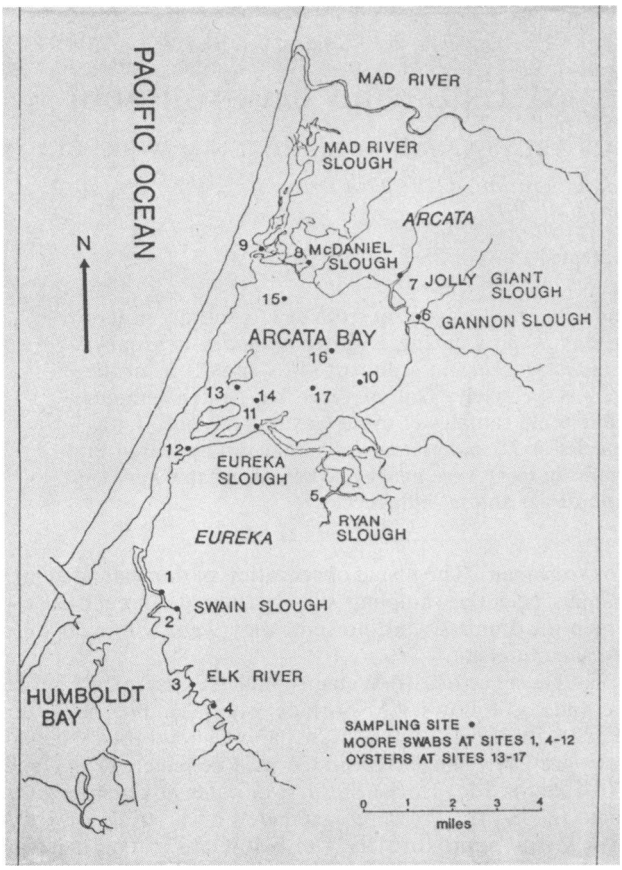

FIG. 1. Map of Humboldt-Arcata Bay area sampling sites. Fresh water, sites 1 to 9; sediment, sites 1 to 5 and 8; seawater, sites 10 to 12; and oyster, sites 13 to 17.

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,

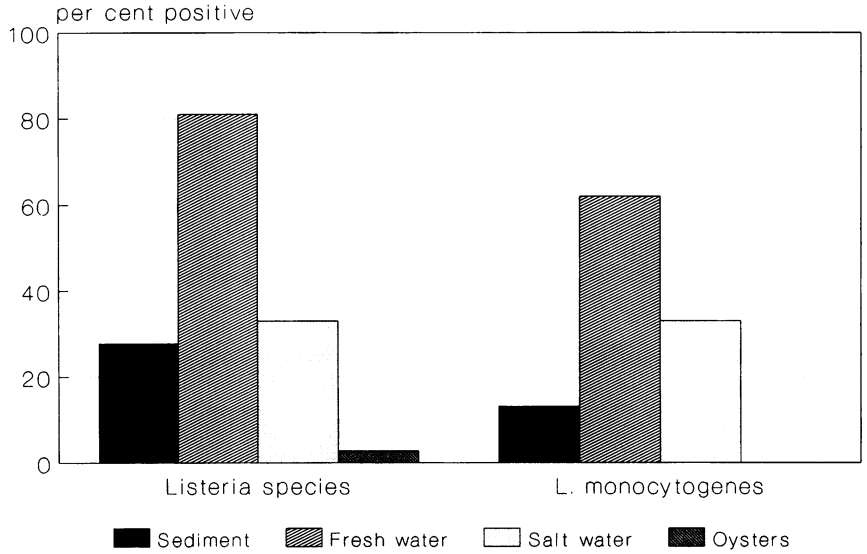

FIG. 2. Incidence of total *Listeria* species and *L. monocytogenes* by sample type.

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.

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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.