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The isolation of *Listeria* species from fresh-water sites in Cheshire and North Wales

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SUMMARY

We report a study to determine the presence of *Listeria* species in surface waters. One hundred ml volumes of 30 water samples taken from 21 different sites were analysed. Most of the samples examined were from ponds and lakes. *Listeria* species were isolated on eight (27%) occasions, six of these isolates were *L. seeligeri*, one was *L. innocua* and one *L. welshimeri*. Although not statistically significant, coliform and *Escherichia coli* counts were higher in waters that were positive than were negative for *Listeria* spp. It is suggested that the low isolation rate of listeria in this study reflects the fact that most waters examined did not receive sewage outfalls. Water sports activities are unlikely to be a risk factor for listeriosis.

INTRODUCTION

That listeriosis can be a food-borne disease has been highlighted by several recent food poisoning outbreaks and its isolation from a wide variety of foods [1–4]. *Listeria* spp. have also been isolated from both domestic and wild animals [4–6]. The suggestion has been made that *Listeria* spp. can survive for long periods in the environment [7, 8]. What role environmental contamination plays in the epidemiology of disease, either from infection of food animals or of humans directly is unclear. This study set out to determine whether surface fresh waters could act as a potential reservoir of infection.

MATERIALS AND METHODS

Water was sampled from 21 sites within Cheshire and North Wales by Environmental Health Officers and submitted to the laboratory for testing within 6 h. Five of 30 samples taken from these 21 sites were canal water, three were river water and the remaining were from ponds and lakes.

One hundred ml samples were filtered through a 0·45 μm membrane, which was placed in 225 ml of buffered peptone water and incubated at 21 °C. After 24 and 72 h incubation 10 ml of the buffered peptone water was added to 30 ml of listeria enrichment broth [9]. The listeria enrichment broth was subcultured onto acriflavine-ceftazidime agar (CM856, Oxoid) and incubated further for 24 h at 30 °C. One colony of each colonial type was tested for aesculin hydrolysis. Colonies

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Table 1. *Isolation of* Listeria *species from surface fresh waters**

Site	Description of site	No. of samples	No. positive	Identity
A	Fresh water dock	4	1	*L. seeligeri*
B	Canal	1	1	*L. innocua*
C	River	2	1	*L. welshimeri*
D	River	1	1	*L. seeligeri*
E	Pond	1	1	*L. seeligeri*
F	Lake	1	1	*L. seeligeri*
G	Lake	1	1	*L. seeligeri*
H	Pond	1	1	*L. seeligeri*

* 18 further samples from 13 ponds and lakes were negative, one site sampled four times and two sites sampled twice.

showing aesculin hydrolysis were further identified according to the scheme described previously [1].

On most of the samples *Escherichia coli* and coliform counts were also done [10]. The water samples were diluted in $\frac{1}{4}$ strength Ringer's solution and 100 ml volumes of undiluted sample and 1 : 10, 1 : 100 and 1 : 1000 dilutions filtered through 0·45 μm nitrocellulose membranes in duplicate. Both filters of each dilution were incubated at 30 °C for 4 h on membrane lauryl sulphate broth [10], then the coliform count filter was incubated at 37 °C and the *E. coli* filter at 44 °C for a further 14 h. Coliforms were identified as yellow colonies on the 37 °C filter that produced acid and gas from lactose at 37 °C, grew in the presence of bile salts and were oxidase negative [10]. *E. coli* were yellow colonies on the 44 °C filter which gave a positive Eijkman reaction [10].

RESULTS

Of the 30 samples examined, *Listeria* species were isolated on 8 occasions (27 %), or 38 % of the 21 sites. Six of these eight positive isolations were identified as *L. seeligeri*, one as *L. innocua* and one as *L. welshimeri*. Table 1 lists the positive samples and their origin. Only 24 of the 30 samples had coliform counts performed, 5 of the listeria positive samples and 19 of the listeria negative samples. Both *E. coli* and coliform counts were higher in the listeria positive samples than in the negative samples (Table 2). However, using the Mann–Whitney U test, these differences were not statistically significant due to the small numbers included in the study.

RESULTS

Previous studies of listeria contamination rates of surface waters have tended to concentrate on water that was contaminated with sewage treatment effluents. Watkins and Sleath reported isolating *L. monocytogenes* from all of nine river waters examined [11]. Dijkstra reported isolating *L. monocytogenes* from 21 % of a variety of surface waters in the north of the Netherlands, noting higher contamination rates (67 %) in waters near sewage treatment plant effluents [12]. However, the methods used would not have been able to distinguish between *L. monocytogenes* and any of the other, currently known, *Listeria* species.

Table 2. *Median, mean, minimum and maximum* E. coli *and coliform counts in listeria positive and negative water samples*

	E. coli		Coliform	
Counts per 100 ml	Listeria positive	Listeria negative	Listeria positive	Listeria negative
Median	1500	110	4500	700
Mean	2600	515	7200	1880
Minimum	< 10	40	230	18
Maximum	8000	3300	24000	10000

There have been some studies done after the taxonomy of listeria was more adequately defined. Colburn and co-workers reported isolating *Listeria* species from 81% of river waters in California [13]. They also found that *L. monocytogenes* was the most frequently isolated species, being present in 62% of water samples. The authors suggested that there was a correlation between the isolation of *L. monocytogenes* from a water and the potential that water was contaminated with bacteria of faecal origin from farming or municipal sources. Another study found that 92·5% of *Listeria* spp. isolated from municipal waste water were *L. monocytogenes*, whilst 4·2% were *L. innocua* and 3·3% were *L. seeligeri* [14]. Luppi and colleagues isolated *Listeria* spp. from 11 of 50 (22%) of water samples from the river Pô [15]. They isolated *L. monocytogenes* once, *L. innocua* seven times, *L. seeligeri* twice, and *L. welshimeri* once.

We found *Listeria* species present in water samples less often than in some of the above studies [11, 13], but at about the same rate as others [12, 15]. Whilst we examined only 100 ml volumes, studies that have quantitated the presence of *Listeria* spp. in water found counts sufficiently high to be detected by this lower volume [11, 16]. The most probable explanation for the lower isolation rate in this study was that the waters examined tended to be relatively free from faecal contamination and did not receive sewage effluents. Indeed, the majority of the waters included in our study were from lakes and ponds being used for recreational purposes. Two of three river waters examined in our study were positive. The suggestion the listeria is more likely to be isolated from faecal contaminated waters is further suggested by the observation that *E. coli* and the coliform counts were higher in the listeria positive samples.

The major difference between our study and all previous studies was the finding that *L. seeligeri* was the most frequent isolate. *L. seeligeri* is isolated from clinical material only very rarely and uncommonly from animal specimens [1, 17]. By contrast, one previous study has shown it to be the most common *Listeria* sp. from environmental sources [17]. *L. seeligeri* was the only *Listeria* spp. isolated from the 22 still waters in this study (ponds and lakes).

The available evidence suggests that the main ecological niche of *L. monocytogenes* is the intestinal tract of man and animals, whilst that of *L. seeligeri* is the environment [1, 17]. This study would suggest that environmental contamination from fresh waters, unpolluted by sewage effluent, is unlikely to play a major role in the epidemiology of human listeriosis. Whether the primary ecological niche of *L. seeligeri* is the aquatic environment, or whether our findings

represent contamination of fresh waters from other environmental sources, is unclear.

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