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# *Listeria* species in domestic environments

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## SUMMARY

Using a direct isolation method *Listeria* spp. were detected in 101 (47·4%) of 213 houses investigated. *L. monocytogenes* was present in 45 houses (21·1%). *Listeria* spp. occurred at all sampling sites. Dish-cloths (37%) and surface samples round the drain in the bathroom (27·2%) were most frequently contaminated. Highest numbers (*c.* $10^4$ c.f.u./object) were found in dish-cloths and washing-up brushes. Lower levels (up to $10^3$ c.f.u./object) were obtained from kitchen sinks, refrigerator vegetable compartment samples and tooth brushes. In total, 132 isolations of *Listeria* spp. were made from 871 samples. *L. innocua* (53%) and *L. monocytogenes* (41%) were the predominant species in the positive samples. Other *Listeria* spp. were found in only 6% of the positive samples.

## INTRODUCTION

The genus *Listeria* consists of six species: *L. monocytogenes*, *L. ivanovii* subspp. *ivanovii* and *londoniensis*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, and *L. grayi* [1]. Only *L. monocytogenes* has been associated with outbreaks of human foodborne illness. Although its pathogenicity to animals and workers in contact with diseased animals has been known for many years, the organism was recognized as a foodborne pathogen only after several major outbreaks [2]. Improvements in the isolation and subsequent identification techniques of *Listeria* spp. have extended knowledge of its ubiquity in the environment [3].

*L. monocytogenes* has been isolated from an extensive range of raw and processed food products including paté and other cooked meat products [4], soft cheeses [5], (smoked) fish products [6, 7] and vegetables [8]. When this pathogen is isolated from processed foods, it is usually not the result of inadequate heat processing, but rather post-process contamination of the product. In most cases, *L. monocytogenes* can be isolated from the environment

of food plants which have produced listeria contaminated product [3, 9]. In food factories, listerias were found in drains, floors, standing water, residues and food contact surfaces in descending order of frequency. In dry culinary food units no samples were found to be contaminated, suggesting that dry conditions and the restriction of food residues contribute to the control of these organisms [10].

Although various food products have been implicated in outbreaks of listeriosis, the sources and routes of infection are usually unknown. The investigation of a case of listeriosis, whether sporadic or in the context of an epidemic, is often hampered both by the limitations of traditional typing techniques and by the occurrence of strains unrelated to the outbreak strain in implicated food products and in the environment [11]. This can be contributed to the isolation methods used. During enrichment, the presence of the strain isolated from patients may be masked by faster growth of other *Listeria* spp. present in the sample [12, 13, 14]. It cannot be ruled out that unknown sources of *L. monocytogenes* may also be responsible for illness. Although presence of *Listeria*

spp. in household environments has been demonstrated [10], domestic households have not often been included in epidemiological studies. The isolation of listerias from such environments raises the possibility that humans may be exposed regularly to these organisms from sources other than contaminated food.

In this study the occurrence of *Listeria* spp. was investigated by sampling six domestic environments in 213 households in The Netherlands.

## MATERIAL AND METHODS

### Growth experiments

Appropriate dilutions in Peptone Physiological Saline Solution [PPSS; composition: NaCl ($8.5 \text{ g l}^{-1}$) and Neutralized Bacteriological Peptone (Oxoid L34, $1 \text{ g l}^{-1}$)] of an overnight culture (20 h at 37 °C) of *L. monocytogenes* (isolated from a dish-cloth) in Brain Heart Infusion Broth (BHIB, Difco 0037-01-6) were used to inoculate dish-cloths, washing-up brushes and toothbrushes (*c.* $10^3$ cells/object). The inoculum level was checked by plating on Tryptone Soya Agar (TSA, Oxoid CM 131). To check recovery, survival and growth of *Listeria monocytogenes*, objects were investigated 30 min after inoculation and after 24 h incubation at room temperature (without any covering), according to the procedure described under enumeration of *Listeria* spp.

### Sampling sites and methods of sampling

Samples were taken from 6 domestic environments in 213 randomly selected Netherlands households. The environments sampled in each household comprised dish-cloths, washing-up brushes, toothbrushes, the bathroom (shower), the kitchen sink and the vegetable compartment of the refrigerator. Since low numbers of listerias probably play a minor role in contamination and infection, a direct (selective) plating procedure was used [15].

Surface samples ($20 \text{ cm}^2$) were taken in bathrooms, from the kitchen sink (both sites round the drain) and from the bottom of the vegetable compartment in refrigerators. For each area two swabs (Falcon, 36/2096) were used. Using a swab moistened in PPSS with the addition of 0.1 % Tween 80 (Merck 822187) (PPSST), the surface was sampled with a rotary movement, at first in a north/south direction, followed by an east/west movement. Then the surface was sampled in the same way with a dry swab. Both swabs

were placed in a tube with 3 ml PPSST. All households were asked for dish-cloths, washing-up brushes and toothbrushes, which were packed in sterile stomacher bags. All samples were transported in melting ice in insulated cool boxes, to the laboratory and investigated within 2 h. The type of house, number of residents and time and day of sampling were listed.

### Enumeration of *Listeria* spp.

Tubes with swabs were mixed thoroughly by Vortex mixing (1 min). One ml PPSST was distributed onto three Enhanced Haemolysis Agar plates (EHA) [16] and from a tenfold dilution in PPSS, 0.1 ml was transferred in duplicate to EHA plates. Since large numbers of *Bacillus* spp. could be expected in samples, nalidixic acid (Sterling NL-85/50; $20 \text{ mg l}^{-1}$) was added as a filter sterilized solution in distilled water:NaOH (60 %:40 %) to the EHA medium to inhibit these organisms.

PPSST was added to dish-cloths and washing-up brushes (50 ml) and to toothbrushes (15 ml). Stomacher bags were mixed vigorously by shaking (1 min). From each sample 1 ml was distributed onto three EHA plates and 0.1 ml from a tenfold dilution in PPSS was transferred in duplicate to EHA plates.

EHA plates were incubated for 2 days at 37 °C. *Listeria* spp. were identified by fluorescence under UV light (366 nm); in addition *L. monocytogenes* showed haemolysis. Typical listeria colonies (three from each plate) were purified by streaking onto a non-selective medium (TSA) and incubated for 2 days at 30 °C. Colonies demonstrating a characteristic blue colour under Henry illumination were subjected to confirmatory testing, using Gram reaction (Gram-positive bacilli or coccobacilli), motility (umbrella type), presence of catalase (+) and oxidase (−) [17] and haemolytic activity (+ or −) [18]. Colonies demonstrating these characteristics were transferred to TSA slants, incubated for 2 days at 30 °C and further identified using API *Listeria* identification strips (bioMérieux, Lyon, France) according to the manufacturer's instructions [16].

## RESULTS

Recovery of listerias from the samples by the proposed procedure, was checked first using artificially contaminated samples. With the methods described we were able to isolate listerias (recovery 75–120 %).

Table 1. *Occurrence of* Listeria *spp. in domestic environments*

| Type of sample (No.) | Listeria Number present (%) | Isolated *Listeria* spp. | | |
|---|---|---|---|---|
| | | *innocua* | *monocytogenes* | Other spp. |
| Dish-cloth (108) | 40 (37) | 20 | 18 | 1 (*seeligeri*) |
| | | | | 1 (*welshimeri*) |
| Washing-up brush (96) | 12 (12·5) | 6 | 6 | — |
| Kitchen sink* (210) | 12 (5·7) | 8 | 3 | 1 (*grayi*) |
| Refrigerator*† (204) | 8 (3·9) | 3 | 5 | — |
| Bathroom* (206) | 56 (27·2) | 30 | 21 | 1 (*ivanovii*) |
| | | | | 1 (*grayi*) |
| | | | | 2 (*seeligeri*) |
| | | | | 1 (*welshimeri*) |
| Toothbrush (47) | 4 (8·5) | 3 | 1 | — |
| Total (871) | 132 (15·2) | 70 | 54 | 8 |

\* Swab samples (20 cm²).
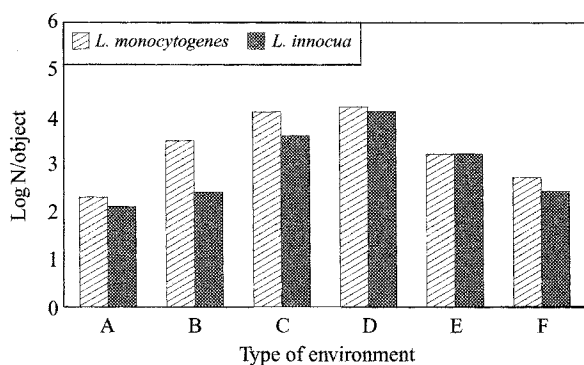† Vegetable compartment.



**Fig. 1.** Mean values of *Listeria* spp. in domestic environments (log N/object). A, vegetable compartment (surface sample, 20 cm²); B, kitchen sink (surface sample, 20 cm², round the drain); C, washing-up brush; D, dish-cloth; E, toothbrush; F, bathroom (surface sample, 20 cm², round the drain).

After 1 day at room temperature (20 °C) a slight increase in numbers was observed, particularly in used objects (data not shown).

The occurrence of *Listeria* spp. at various sampling sites is presented (Table 1). From 871 samples 132 listerias were isolated. *L. innocua* (53 %) and *L. monocytogenes* (41 %) were the predominant species in the positive samples. Other *Listeria* spp. were found in only 6 % of the positive samples. Strikingly, there were no samples in which more than one species was detected. The mean contamination levels for *L. innocua* and *L. monocytogenes* (logN/object) are given (Fig. 1). Highest counts were found on dish-cloths, washing-up brushes and toothbrushes ($10^3$–$10^4$ c.f.u./object). Lower numbers ($10^2$–$10^3$ c.f.u./object) were detected in the bathroom, the kitchen

sink and in the refrigerator vegetable compartment. Mean contamination levels for the other *Listeria* spp. were comparable with those observed for *L. innocua* and *L. monocytogenes* (data not shown).

For samples from the bathroom, washing-up brushes and dish-cloths, a relative frequency distribution is given (Figs 2 a–c). In 70 % of the positive samples from the bathroom and in all samples from washing-up brushes and dish-cloths numbers of *L. monocytogenes* were higher than $10^2$ c.f.u./object. In about 80 % of the positive dish-cloths and washing-up brushes, this pathogen was present in numbers > $10^3$ c.f.u./object.

Various types of accommodation were investigated including detached villas, terraced houses, apartments and student apartments ($n = 213$). The level of contamination of the samples was similar in the different types of housing. In 101 of the 213 houses examined (47·4 %) *Listeria* spp. were detected. In 74 of the 101 houses (73 %) listeria was isolated from only 1 of the 6 samples investigated. In 23 houses (23 %) listerias were present in 2 samples and in 4 houses (4 %) listeria occurred in 3 of the samples analysed (data now shown). Neither the number of residents in a house (1–9), nor the time of sampling (morning or afternoon) influenced the contamination rate of the sampling sites (data not shown).

## DISCUSSION

Since *Listeria* spp. are commonly found in the general environment [19], the presence of these organisms in the domestic environment is not surprising. In
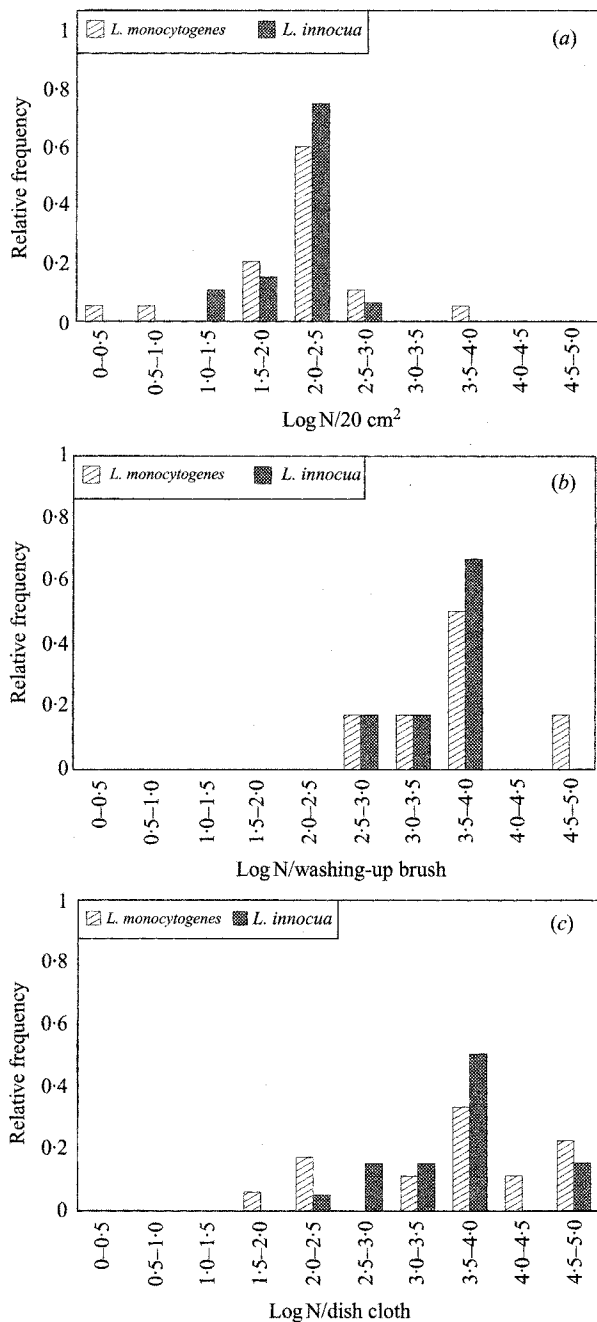
**Fig. 2.** (a) Relative frequency distribution of *Listeria* spp. in the bathroom (surface samples round the drain). (b) Relative frequency distribution of *Listeria* spp. on washing-up brushes. (c) Relative frequency distribution of *Listeria* spp. on dish-cloths.

previous work 20% of kitchens in 35 households were found to be contaminated with listerias. If substrates are available, almost any moist environment will probably support these and other micro-organisms [10]. However, the extent to which products or people become contaminated will depend, amongst other factors, on the numbers of micro-organisms present. Unfortunately, most studies detecting pathogens

employ enrichment protocols. Therefore little quantitative information is available in the literature.

From the experiments with artificially contaminated dish-cloths, toothbrushes and washing-up brushes, it was shown that with the methods we used 75–120% of the initial flora could be recovered. The relatively high standard deviations (up to 26%) may be due to the isolation technique used. Vigorous shaking of stomacher bags by hand is probably less effective than the use of mechanical equipment. On all objects the test strain was detectable after 24 H at room temperature. On new objects numbers of *L. monocytogenes* were slightly lower when compared with the numbers on old objects. This might have been due to lack of nutrients or to the presence of inhibitory substances [20]. The presence of growth, albeit slight, indicates that in the case of bad hygienic practices, outgrowth to high numbers could occur.

Table 2 shows that *Listeria* spp. were isolated from 101 (47·4%) of 213 households investigated. In 45 (21·1%) houses *L. monocytogenes* was present. Although this is an increase in comparison with the data of Cox and colleagues [10], it remains uncertain if this was due to the increase in sampling sites, in the numbers of houses investigated, or to differences in isolation protocols. Since in this study isolation of *Listeria* spp. was performed using a direct (selective) plating procedure, other listeriae and competing micro-organisms could not mask the presence of *L. monocytogenes* [14]. Different *Listeria* spp. were isolated from one household, but in positive samples only one *Listeria* sp. was detected. Confirmation and subsequent identification of more than three typical colonies on the plates might have led to the detection of more *Listeria* spp. in the positive samples. However, it is likely that growth of *Listeria* at the sampling sites is comparable to growth in enrichment broths. Fast growing listerias (adapted to the ecological niche) will mask the presence of slow growing species.

Highest contamination levels were found in wet environments such as dish-cloths (37%) and in surface samples from the bathroom (27·2%). However, samples taken from round the drain in the kitchen sink had a significant lower contamination level (5·7%). This may be partly due to the type of material, stainless steel for the kitchen sink and tiles in the bathroom. In addition, kitchen sinks are more often in contact with warm or hot water containing detergents (washing-up liquids), which may drastically reduce numbers of listerias present.

Washing-up brushes and toothbrushes will be

Table 2. *Presence of* Listeria monocytogenes *and* Listeria innocua *in samples from different types of houses*

| Type of house (No.) | Number of samples | *Listeria* spp. (%) | *L. monocytogenes* (%) | *L. innocua* (%) |
|---|---|---|---|---|
| Detached villa (17) | 96 | 14 (14·6) | 9 (9·4) | 5 (5·2) |
| Terrace house (75) | 271 | 40 (14·8) | 13 (9·4) | 23 (8·5) |
| Apartment (54) | 217 | 40 (18·4) | 15 (6·9) | 25 (11·5) |
| Student apartment (67) | 287 | 38 (13·2) | 17 (5·9) | 17 (5·9) |
| Total (213) | 871 | 132 (15·2) | 54 (6·2) | 70 (8) |

wetted on only a few occasions each day. Growth of listerias could occur during these brief periods, depending on the type and the corrosion (cracks) of the material, and the presence of any organic matter. Growth would be likely to stop after most of the water had evaporated. This probably accounts for the lower contamination level of washing-up brushes and toothbrushes compared with dish-cloths.

Listerias were detected in the vegetable compartment of only 3·9% of the 204 refrigerators investigated. Cox and colleagues obtained similar results (1 positive from 35 refrigerators sampled) [10], whereas this pathogen was not isolated by Jackson and colleagues [21]. A possible explanation for the low contamination rates of vegetable compartments in refrigerators maybe that these parts of the refrigerator are likely to be cleaned frequently since they often become visibly soiled. Moreover, the growth of the organisms may be limited by the low relative humidity as a result of the removal of water from the air by condensation and freezing. It is reported that on inert and dry surfaces at room temperature, the number of viable *L. monocytogenes* cells decreased. However, the organisms could be recovered for 4–6 weeks [22, 23].

This study demonstrates that *L. monocytogenes* and *L. innocua* are the predominant *Listeria* spp. in domestic environments. All listerias, including *L. monocytogenes*, were commonly found in high numbers in wet places. The occurrence of listerias in high numbers suggests that the organisms may be growing actively and that hygienic practices in domestic kitchens could be improved. Humans are exposed to this pathogen on a regular basis, because of its ubiquity in food products and the environment [2]. However, there is as yet no evidence that domestic sources of *L. monocytogenes* play a role in the contamination of foods.

In previous studies on microbial contamination in the domestic environment [24, 25], dish cloths were noted as being sources of contamination with *Entero-*

*bacteriaceae* in approximately 45–50% of homes examined. From our data it becomes clear that *L. monocytogenes* may be present in high numbers in the domestic environment. Even low number of *L. monocytogenes*, may proliferate and become hazardous if transferred to food products with a long (refrigerated) shelf-life. Other workers have recognized the potential for spread of microbial contamination via cleaning utensils and the potential for some micro-organisms to persist in the environment [26–28].

Outbreaks of listeriosis have been associated frequently with foods; if future epidemiological investigations also included the domestic environment, it would help to clarify the relative importance of these sources in contributing to outbreaks of listeriosis.

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