Listeria in effluents from the food-processing industry

A. SCHÖNBERG and K. GERIGK *

Summary: There is general agreement that listeriosis has a significant impact on Man as well as on animals. Listeria monocytogenes has been isolated from the faeces of healthy human and animal carriers and from various environmental sources. L. monocytogenes is the pathogenic species most responsible for abortion, septicemia and meningitis in animals and Man. Listeria ivanovii is a primary cause of abortion in animals. Owing to a number of epidemics and single cases caused by food contaminated with L. monocytogenes, listeriosis has received more attention in the past ten years than ever before.

Entry of the organism into food-processing plants is primarily caused by animals which excrete Listeria in their faeces. Other sources of entry are raw foods of animal origin and personnel in food establishments.

Proliferation of Listeria is promoted by high humidity and nutrient waste in certain food production plants. Removal of Listeria is almost impossible by routine disinfection. Listeria-contaminated sites pose a serious risk of recontamination of food-processing equipment and processed foods. Moreover, such sites represent an inexhaustible source of entry for Listeria in plant effluents.

There is no denying that effluents from food-processing plants increase the spread of Listeria in the environment. However, considering the existence of other sources of entry, such as human and animal husbandry wastes, and that circulation and recontamination within the environment itself are also possible, this may not be a particularly important risk.

KEYWORDS: Effluents - Environment - Food-processing industry - Listeria - Man.

INTRODUCTION

There is general agreement that listeriosis has a significant impact on animals and Man. First described in England in 1926, Listeria monocytogenes was identified as the cause of mononuclear leucocytosis in laboratory rabbits and remained the only recognised species of this genus during the next two decades (32). Through the use of highly selective media, numerous strains which appeared to be “atypical” when compared to L. monocytogenes were isolated from the faeces of healthy human and animal carriers and from various environmental sources (35). The genus Listeria comprises seven species.

* Institute of Veterinary Medicine, Robert von Ostertag-Institute, Federal Health Office, P.O. Box 330013, D-1000 Berlin 33, Federal Republic of Germany.
L. monocytogenes is the major pathogenic species responsible for abortion, septicaemia and meningitis in animals and Man. L. ivanovii mainly causes abortion in animals (ewes, cows and goats). The non-pathogenic species are L. innocua, L. welshimeri, L. seeligeri, L. grayi and L. murrayi. The pathogenic species can be differentiated from others by their haemolytic properties and by biochemical tests (Table I) (36). Listeria is a small mesophilic Gram-positive rod; cells occur singly or in short chains, which are sometimes coccoid. The bacterium is motile when cultured at 20°C-25°C. It grows at temperatures which range from 0°C-45°C (optimal growth 30°C-37°C) and at pH values of 5.0 to 9.6. Listeria multiplies on rather simple nutrient media (23, 40). Selective media have been developed to cultivate Listeria from materials contaminated by different bacteria.

### Table I

**Biochemical differentiation of Listeria species**

<table>
<thead>
<tr>
<th>Species</th>
<th>Beta Hemolysis</th>
<th>CAMP-test with R. equi</th>
<th>D-Xylose</th>
<th>L-Rham-nose</th>
<th>Alpha-methyl D-Mannoside</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. monocytogenes</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L. ivanovii</td>
<td>+ + +</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L. innocua</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+ / -</td>
<td>-</td>
</tr>
<tr>
<td>L. welshimeri</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+ / -</td>
<td>+</td>
</tr>
<tr>
<td>L. seeligeri</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Serologically, L. monocytogenes and related species can be subdivided into 16 serovars. Most cases of disease are caused by one of three serovars (1/2a, 1/2b or 4b). Phage typing is possible when carried out in specialised laboratories for epidemiological studies. Listeriosis has received increasing attention during the last ten years due to a number of epidemics and single cases caused by foods contaminated with L. monocytogenes (23, 38, 45). Foodborne outbreaks with 30% case-fatality rates have occurred in North America due to coleslaw (Nova Scotia, 1981) and soft cheese (Los Angeles, 1985). Further outbreaks were reported as a result of intensified investigations, one of which demonstrated that the outbreak in Switzerland at the end of 1987 had been caused by soft cheese (38, 46).

Listeriosis in Man is a relatively rare disease. In the 1950s, it was assumed that animals played an important role in the transmission of the disease to Man. A review of the human cases, however, has failed to demonstrate a correlation between animal and human listeriosis (22).

Three major outbreaks of human listeriosis in which foods were identified as vehicles of infection occurred in the early 1980s (8). Human waste is another source of Listeria. Studies in several publications have shown that even clinically healthy people may excrete L. monocytogenes in their faeces whether or not they have had contact with animals. Of the investigated samples, including those from pregnant women, 1-77% proved to be Listeria-positive (4, 23, 25, 27).
The Office International des Epizooties (OIE) has compiled two lists of animal diseases so that Member Countries may submit notifications. Listeriosis belongs to neither the List A category of highly contagious diseases nor to List B of transmissible animal diseases with important socio-economic health implications (6).

Several research workers have demonstrated the occurrence of *Listeria* in the environment, soil, plants, sewage and municipal waste. Overall contamination rates of samples with *Listeria* range from 10 to 44% in plants and from 13 to 51% in soil. More precisely, the respective contamination rates of *Listeria* in plants and soils are as follows: 10% in crops and 13% in corn field soil; 13 and 14% in grain fields; 13 and 19% in cultivated fields; 44 and 51% in uncultivated fields; 16 and 9% in meadows and pastures; 21 and 15% in forests; and 23 and 43% on wildlife feeding-grounds (44).

**LISTERIA IN FOOD-PROCESSING INDUSTRIES**

**Animals**

Table II shows a variety of animals from which *L. monocytogenes* has been isolated. This list primarily includes birds and mammals, especially farm animals, pets and rodents; however, amphibia, fish and insects can also harbour *Listeria* (7).

**TABLE II**

*Animals from which L. monocytogenes has been isolated*

<table>
<thead>
<tr>
<th>Authors</th>
<th>Mammals</th>
<th>Miscellaneous</th>
<th>Birds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gitter (1985)</td>
<td>Cattle, sheep, goats,</td>
<td>Fish</td>
<td>Canaries, chickens</td>
</tr>
<tr>
<td></td>
<td>swine, horses, dogs,</td>
<td></td>
<td>cranes, doves, ducks, eagles,</td>
</tr>
<tr>
<td></td>
<td>chinchillas</td>
<td></td>
<td>geese, lorikeets, parrots,</td>
</tr>
<tr>
<td>Gray &amp; Killinger</td>
<td>Cats, deer, racoons,</td>
<td>Crustaceans</td>
<td>partridges, pheasants, pigeons,</td>
</tr>
<tr>
<td>(1966)</td>
<td>guinea pigs, lemmings,</td>
<td></td>
<td>turkeys, white grouse,</td>
</tr>
<tr>
<td></td>
<td>skunks</td>
<td></td>
<td>whitethroats, woodgrouse</td>
</tr>
<tr>
<td>Bacon &amp; Miller (1958)</td>
<td>Rabbits, voles, gerbils</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Botzler &amp; Cowan (1985)</td>
<td>Frogs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kratokhuiil (1953)</td>
<td>Ticks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weis &amp; Seeliger (1975)</td>
<td></td>
<td></td>
<td>Chaffinches, hawks, sparrows</td>
</tr>
<tr>
<td>Fenlon (1985)</td>
<td></td>
<td></td>
<td>Seagulls</td>
</tr>
</tbody>
</table>
The infection in ruminants is of particular importance. Silage of poor quality and with a rather high pH level is the principal source of this infection; 40% of the samples taken from the silage of farms where abortion in cattle had occurred were found to be Listeria-positive. Worthy of note is that abortion in cattle and sheep occurs soon after they have started to feed on silage. Approximately 70% of aborting cows were observed to suffer from retained placenta and vaginal discharge, resulting in the spread of Listeria into the environment (13). Approximately 25% of infected cows excreted L. monocytogenes in their faeces for three to twelve months after abortion. The milk of 16% of these cows was contaminated for one to twelve months; however, inflammation of udder tissue was so light that no changes in the milk were visible. In 45% of infected cows, the number of Listeria in milk occurred in such small amounts that isolations were only possible after enrichment (13, 37). In addition, approximately 20% of sheep from infected flocks were found to excrete L. monocytogenes in their faeces (4).

Listeria in many species is found not to cause illness. Listeria have been isolated from 10 to 15% of faecal samples from clinically healthy cattle (5, 24, 27, 41, 44). Approximately 2% of faecal samples from clinically healthy sheep were reported positive for Listeria (4). In another study, faecal samples collected from poultry and cattle harboured L. monocytogenes in 33 to 51% of these cases (41).

Broilers may excrete L. monocytogenes in their faeces and thus contaminate litter, environment, other animals and even Man (21, 28). In one study, Listeria was found in approximately 24% of the examined broiler houses. After disinfection, 17% of the broiler houses became re-infected, some as often as eight times in succession (11).

In two studies on wildlife, L. monocytogenes was isolated from deer (7-11%), foxes (14%), badgers (30%), hares (27%) and birds (17%) (15, 43).

**Food-processing industries**

The organism enters food-processing plants mainly by animal excretion of Listeria in faeces. Raw foods of animal origin and other raw products used for the processing of foods are additional sources of entry. Furthermore, unhygienic handling procedures by staff may spread contamination and cause Listeria to enter the food-processing environment.

Proliferation of Listeria is promoted by high humidity and nutrient waste in certain food production plants. Slaughterhouses, dairies and meat-processing plants offer particularly favourable conditions for the survival, growth and multiplication of Listeria. Despite regular cleaning and disinfection of food-processing facilities and equipment, the organism is able to survive in highly inaccessible places, such as drains, where total removal of Listeria is impossible. Such Listeria-contaminated sites pose a serious risk of recontamination of food-processing equipment and processed foods; they also represent an inexhaustible source of entry of Listeria into plant effluents.

Listeria infiltration of the food-processing chain can be demonstrated by the widespread occurrence of the organism in processed products. L. monocytogenes was isolated from poultry at a rate of 60 to 85% (34, 39). Meat and meat products contained L. monocytogenes in 10 to 34% of samples (31, 33, 39), while such dairy products as pasteurised milk and soft cheese harboured Listeria in concentrations significant enough to cause illness in Man (16, 29). The organism could be isolated from raw fish and fish products in 37% of the cases studied (31).
The effluents of food-processing plants may also be heavily contaminated with *Listeria*. Out of 54 rinse-off samples from a poultry slaughter plant, 24 proved to be positive for *Listeria* (17). *Listeria* species were present in 13.4-18.8% of plucker drip, 6.7-12.5% of chiller overflow and in 33-37.5% of recycled water used for cleaning gutters (19, 20). In another study, *L. monocytogenes* was detected in 1.7% of the samples from the equipment in a slaughter plant, although the organism was not found in waste water (9).

**Table III**

**Relative importance of different environments as sources of *Listeria* in food-processing units**

(10)

<table>
<thead>
<tr>
<th>Sample description</th>
<th>Total(1)</th>
<th><em>Listeria</em> spp. + (2)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drains</td>
<td>66</td>
<td>36</td>
<td>54.5</td>
</tr>
<tr>
<td>Condensed/stagnant water</td>
<td>43</td>
<td>20</td>
<td>46</td>
</tr>
<tr>
<td>Floors</td>
<td>79</td>
<td>36</td>
<td>45</td>
</tr>
<tr>
<td>Residues</td>
<td>97</td>
<td>32</td>
<td>32.9</td>
</tr>
<tr>
<td>Process equipment</td>
<td>104</td>
<td>20</td>
<td>19.3</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>219</td>
<td>37</td>
<td>16.9</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td>608</td>
<td>18</td>
<td>29.8</td>
</tr>
</tbody>
</table>

1) Total samples examined, all positive factories
2) Total samples positive for *Listeria* spp., all factories

Altogether, investigations conducted both in food-processing and non-food industrial environments showed that samples from drains, floors and water residues, as well as from surfaces in contact with foods, contained *Listeria* relative to the frequency of contamination (10). Table III summarises the results obtained from all the factory environments studied. The highest isolation rate, 54.5%, was observed in samples from drains; approximately 85% of the 34 positive samples contained the organism in amounts of 10-10^4 per gram (Table IV). All drain or residue samples contained *Listeria* in concentrations of at least 10^2/g. However, *L. monocytogenes* was not isolated from samples of two dry culinary food units (10).

The data from food-processing plants demonstrate that measures to control contamination, survival and growth of *Listeria* in the food production environment have limitations. Drains are a permanent reservoir for *Listeria* contamination of effluents. As the number of *Listeria* does not seem to be substantially reduced by biological oxidation during wastewater treatment, the effluents of food-processing plants are capable of contaminating the environment, thereby spreading *Listeria* to farm animals which feed on sludge-treated areas or which come into contact with sewage plant effluents.
**TABLE IV**

*Enumeration of Listeria spp. in environmental samples from ice-cream production* (10)

<table>
<thead>
<tr>
<th>Sample description</th>
<th>Negative on reanalysis</th>
<th>Positive on reanalysis</th>
<th>No. samples positive in $10^n$ g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10^{-1}</td>
<td>10^{-2}</td>
<td>10^{-3}</td>
</tr>
<tr>
<td>Drains</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Floors</td>
<td>-</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Stagnant water</td>
<td>-</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Residues</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Equipment</td>
<td>2</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Totals</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>% of total</td>
<td>8.8</td>
<td>11.8</td>
<td>14.7</td>
</tr>
<tr>
<td>Cumulative %</td>
<td>8.8</td>
<td>20.6</td>
<td>35.3</td>
</tr>
</tbody>
</table>

1) Positive in 25 g
2) Negative in 1 g
3) 2 samples positive in 1 g; 1 sample positive in 17.7 g
4) 1 sample negative in 1 g; 1 sample negative in 0.1 g

**LISTERIA IN EFFLUENTS OF FOOD-PROCESSING INDUSTRIES**

The spread of *Listeria* partly owes to contaminated wastewater effluents. In all of the 33 wastewater samples investigated, *Listeria* was found in concentrations from $10^3$ to $2.4 \times 10^5$/l; 84% of the strains were identified as *L. monocytogenes* (41). In another study of sewage, *L. monocytogenes* was isolated from all samples taken (42). Eight strains of *L. monocytogenes* were isolated by other research workers from 33 samples of urban sewage (30). Samples of effluents taken after biological treatment were examined in two of these studies and *Listeria* was found in all of the samples. The number of organisms had stagnated or increased in 15 of the 33 cases (45%) (18) or remained unaltered over eight weeks (42). *Listeria* was found to accumulate in sludge and to survive for a period ranging from several months to several years (18). A Dutch study showed that effluents of sewage treatment plants had a *L. monocytogenes* contamination rate of 93% (26). In another study, five miles from a treatment plant, the contamination rate was established at 67% (12). Approximately 21% of the surface waters of canals and lakes in the Netherlands proved to be contaminated with *Listeria*. Positive samples were found where the canal flows into the sea, as far as 25 miles from the wastewater plant (12).

The use of sewage products as fertilizer is therefore considered a route of *Listeria* spread in the environment (1, 2). *L. monocytogenes* could be isolated from plants grown on farmland soil which had been treated with sewage sludge, but only in small amounts (5 cells/g) (3) and at a low contamination rate (10%). At the same time,
seagulls feeding at sewage works more often carried *Listeria* in their faeces than those feeding elsewhere (14).

There is no denying that effluents of food-processing plants increase the spread of *Listeria* in the environment. However, in view of such other sources of entry as human and animal husbandry wastes, and considering such factors as circulation and recontamination within the environment itself, this need not be considered a particularly important risk.

***

* * *

**LISTERIA DANS LES EFFLUENTS DES INDUSTRIES DE TRAVERSATION DE PRODUITS ALIMENTAIRES. — A. Schönberg et K. Gerigk.**

**Résumé:** Tous les auteurs s’accordent à reconnaître l’importance des conséquences de la listériose sur la santé publique aussi bien que sur la santé animale. *Listeria monocytogenes* a été isolé dans les fèces de personnes et d’animaux porteurs sains, ainsi que dans l’environnement. *L. monocytogenes* est la principale espèce pathogène responsable de cas d’avortement, de septicémie et de méningite chez l’homme et chez l’animal. *Listeria ivanovii* est responsable d’avortements chez les animaux. La listériose est depuis une dizaine d’années l’objet d’une attention sans précédent, du fait d’épidémies et de cas isolés de toxifi-infections alimentaires provoquées par *L. monocytogenes*.

La pénétration de ces germes dans les usines de transformation agro-alimentaires est due principalement à des animaux excréteurs de *Listeria* dans leurs déjections. Les produits bruts d’origine animale et le personnel de ces établissements constituent d’autres sources de contamination.

La prolifération de *Listeria* y est favorisée par l’humidité ainsi que par la présence des déchets organiques. Il est pratiquement impossible d’éliminer les *Listeria* par les seuls procédés habituels de désinfection. Les locaux contaminés par *Listeria* présentent un risque important de recontamination des équipements utilisés pour le traitement des aliments, ainsi que des produits transformés. En outre, ces locaux constituent une source inépuisable de *Listeria*, répandus par les effluents.

Il est indéniable que les effluents des usines de transformation de produits alimentaires favorisent la propagation des *Listeria* dans l’environnement. Cependant, compte tenu de l’existence d’autres sources d’infection telles que les déchets des élevages, et des possibilités de persistance des *Listeria* dans l’environnement lui-même, il n’est pas certain que le risque présenté par les usines soit de première importance.

**MOTS-CLÉS:** Effluents - Environnement - Homme - Industries de transformation de produits alimentaires - *Listeria*.

**MOTS-CLÉS:** Effluents - Environnement - Homme - Industries de transformation de produits alimentaires - *Listeria*.

**LISTERIA EN LOS EFLUENTES DE LAS PLANTAS DE TRANSFORMACIÓN DE ALIMENTOS. — A. Schönberg y K. Gerigk.**

**Resumen:** La importancia de las consecuencias de la listeriosis para la salud pública y animal es un hecho ampliamente establecido. *Listeria monocytogenes*
ha sido aislado de las heces de personas y animales portadores sanos, así como en el medio ambiente. L. monocytogenes es la principal especie patógena responsable de los casos de aborto, septicemia y meningitis en el hombre y en los animales. Listeria ivanovii causa abortos en los animales. Habida cuenta del número de epidemias y casos aislados de toxo-infecciones por L. monocytogenes, los esfuerzos consagrados a la listeriosis en la última década han sido particularmente importantes.

La penetración de estos gérmenes en las plantas de transformación de alimentos se debe a los animales que eliminan Listeria en sus heces. Otras fuentes son los productos brutos de origen animal y los empleados de estos establecimientos.

El alto grado de humedad existente en las plantas agroindustriales así como los residuos orgánicos favorecen la proliferación de Listeria. Parece imposible eliminar Listeria mediante simples desinfecciones de rutina. Las plantas contaminadas por Listeria presentan un alto riesgo de recontaminación de los equipos de transformación de alimentos así como de los productos tratados. Por otra parte, estas industrias constituyen, a través de los efluentes que producen, una fuente inagotable de Listeria.

No se puede negar que los efluentes de las fábricas de alimentos favorecen la propagación de Listeria en el medio ambiente. Sin embargo, si se considera la existencia de otras fuentes de transmisión, como pueden serlo los residuos de la ganadería, y las posibilidades de sobrevivencia de estos organismos en el medio ambiente, el riesgo representado por estas industrias no parece ser particularmente importante.


**

REFERENCES


