

## Incidence of *Listeria monocytogenes* in Nature

J. WEIS AND H. P. R. SEELIGER\*

*Tierhygienisches Institut, Freiburg i. Br., and Institut für Hygiene und Mikrobiologie der Universität Würzburg, Würzburg, West Germany*

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During a research project on the occurrence of *Listeria monocytogenes* 194 strains were isolated in southern West Germany during the years 1972 to 1974: 154 from soil and plant samples (20.3%), 16 from feces of deer and stag (15.7%), 9 from old moldy fodder and wildlife feeding grounds (27.2%), and 8 from birds (17.3%). The highest number of isolates was obtained from uncultivated fields. The beta-hemolytic serovars 1/2b and 4b were predominant; other serovars (some of them identified for the first time), including nonhemolyzing strains, have been encountered frequently. It is suggested that *Listeria monocytogenes* is a saprophytic organism which lives in a plant-soil environment and therefore can be contracted by humans and animals via many possible routes from many sources.

In spite of the growing literature on *Listeria monocytogenes* the question whether this organism is primarily soil born or originates from animals excreting *L. monocytogenes* with feces (3, 4, 6, 8, 13) remains unresolved. Therefore, this study has focused on the occurrence of *L. monocytogenes* in plants, soil, and feces of wild animals and birds.

### MATERIALS AND METHODS

Samples of plants and soil from various parts of southern West Germany to be tested for *L. monocytogenes* were collected from: (i) cultivated fields (maize, wheat, oats, barley, potatoes, etc. and pastures and meadows), (ii) uncultivated fields (fields and meadows that had lain fallow for years); (iii) forests, wildlife feeding grounds, and mud from creeks, rivers, and ponds); (iv) feces and residues of fodder from wildlife feeding grounds.

Each sample of plant or fodder specimen from wildlife feeding grounds was collected with sterile rubber or disposable plastic gloves or with sterile scissors and was packed in plastic bags. The soil samples were obtained from the surface of the ground, as well as from a depth of 10 cm, using small sterile shovels. It was taken into consideration that soil samples from the surface are regularly contaminated with plant residues and that contamination could not be avoided when soil samples were collected from the depth. Shredded plants, soil samples (15 to 20 g), or feces (1 to 10 g) were suspended in 50 to 400 ml of enrichment broth and grown in 500-ml glass bottles.

The following enrichment media were employed: (i) potassium thiocyanate medium (according to Lehnert [19]) and potassium thiocyanate medium substituted with 10 µg/ml of Acriflavin (Serva) per ml (K. Höhne, Ph.D. thesis, Justus-Liebig University, Giessen, West Germany, 1972); (ii) tryptose broth (Difco); and (iii)

brain heart infusion broth (Difco). For medium (i) the seeded containers were maintained at 22 C for 7 days. After 2 and 7 days, samples were plated on four tryptose agar plates (Difco) with 40 µg of nalidixic acid (Serva, Heidelberg; subsequently called TN medium) added per ml. This TN medium was incubated at 37 C for 24 h and for an additional 24 h at 22 C. Then the growth was checked for typical colonies with a microscope by use of the Henry illumination technique (1, 2). Using enrichment media (ii) and (iii), cultures were maintained for 12 months at 4 C. After 1, 3, 5, and 12 months, one loopful of the material was plated on four TN agar plates.

In addition, a sample from each culture was transferred into Stuart's liquid with the aid of an alginate tampon and incubated at 22 C for 1 week. Subsequently, this tampon was suspended in 10 ml of Ringer solution and one loopful was plated on four TN agar plates (5).

Additionally, brain heart infusion (5.0 ml) was inoculated with 0.1 ml of material from the brain heart infusion enrichment broth, incubated at 22 C for 2 weeks in the dark, and streaked on four TN agar plates (15). All colonies suspected of being *Listeria* were examined for catalase activity and motility and tested further as described by Seeliger (11).

The virulence of the isolated *Listeria* strains was tested in an outbred strain of mice (NMRI/Han) weighing 18 to 20 g. Four mice each were injected with 0.5 ml of an 18-h glucose broth culture intraperitoneally. A strain was considered to be virulent if the mice died within 3 weeks and *Listeria* could be isolated from the organs at necropsy. The determination of serovars was done according to the procedures outlined by Seeliger (11).

### RESULTS

Cultivation in the enrichment broth of Lehnert (9) was found to be an excellent method for

the isolation of *Listeria*. The same medium substituted with acriflavin yielded even better results, but the time of its use was too short for a definite evaluation.

*L. monocytogenes* occurs in a high proportion of plants, soil samples, and feces of deer and stags as well as of birds (Table 1). The highest positive results were obtained from the surface of soil specimens and plants, particularly in fields that had lain fallow for years and were overgrown with grass and small shrubs (Table 2). Only a few strains of *L. monocytogenes* could

be isolated from the depth of soil from uncultivated fields.

Faded and decayed grass was a direct indicator of the presence of *Listeria*. The same *Listeria* serovar was found repeatedly at the same place at half-year intervals. In some instances two different serovars were detected in the same sample. Positive findings were irregular at the same site. From a meadow near Kaiserstuhl that had not been used as pasture land for some years two strains were isolated in the autumn of 1971, none in the summer of

TABLE 1. Occurrence of *L. monocytogenes* and results of the serological analysis of isolated strains

Environment	No. of specimens	% +	No. of strains belonging to serovar										Serovar not determined	
			1/2a	1/2b	1/2c	4b	4c	4d	4f	4g	4*	5		
Cornfields (maize)	71	8.4		2		1	1					1	1	
Grainfields	85	11.8		2	1	3	1							2
Cultivated fields	41	12.2		3	1									1
Uncultivated fields	141	44.0	5	32		11	3		1	2	7	3		1
Meadows and pastures	111	9.9	1	3		6	1				1			
Forests	151	15.2		12	1	3		1	1	1		3		2
Mud	38	31.5	2	4		3					5			
Wildlife feeding grounds	108	23.1	6	7		5	1		3		3			
Residues of fodder from wildlife feeding grounds	33	27.2	1	1	1	2				1	1	2		
Feces	102	15.7	2	1		6	1	1	1	1	2	1		
Birds	46	17.3	2	3		1								2

\* New antigen combinations, not yet designated (see Table 3).

TABLE 2. Incidence of *L. monocytogenes* isolated from plants and soil

Environment	Plants			Soil						
	No.	Positive	%	Surface			Depth (10 cm)			
				No.	Positive	%	No.	Positive	%	
Cornfields	31	3	9.7	23	3	13.0	17			
Grainfields	30	4	13.3	36	5	13.9	19	1		5.2
Cultivated fields	16	2	12.5	16	3	18.7	9			
Uncultivated fields	75	33	44.0	39	20	51.4	27	9		33.3
Meadows/pastures	38	6	15.5	46	4	8.7	27	1		3.7
Forest	61	13	21.3	59	9	15.2	31	1		3.2
Wildlife feeding grounds	39	9	23.1	37	16	43.2	32			

1972, and again two in the autumn of 1972. Somewhat unexpected was the isolation of *L. monocytogenes* from the leaves of shrubs 50 cm above ground, in addition to those on the ground.

*L. monocytogenes* could be isolated from samples of mud in relatively great numbers. It appears to survive and to multiply particularly well in a moist environment. The lowest number of positive results was obtained from fields and meadows used for agricultural purposes.

An entirely different distribution of *L. monocytogenes* was noted in the upper Black Forest, where it was isolated exclusively from the surface of the soil of wildlife feeding grounds. On the other hand, the incidence of *Listeria* in the deciduous forests of the foothills and in the valleys, as well as in the plain, was rather scattered. The low incidence of *L. monocytogenes* in upper Black Forest was associated with low pH values of the local soil (sometimes below 3.5).

A high percentage of samples collected in a small forest north of Salem, near the Lake of Constance, yielded *Listeria*. This forest is surrounded by cultivated fields, and the pH value of the soil samples was found to be between 4.8 to 7.6. In this area *Listeria* was cultured from 22 out of 46 specimens in a single wildlife feeding ground, in its vicinity as well as at a distance of 200 m.

A great number of *Listeria* isolates were obtained from feces and old moldy specimens of fodder collected from wildlife feeding grounds; 17.3% of the birds examined were *Listeria* positive. In a pheasant and a partridge, septicemia was noticed, whereas two blackbirds, a sparrow, a chaffinch, a hawk, and another songbird harbored the organism only in the intestinal tract.

Among the isolated strains, serovars 1/2b and 4b were found to be predominant (Table 1).

The serovars of 20 of the *Listeria* strains (Table 3) belonged to antigen combinations not listed on the extended scheme of Seeliger (12). Six of these strains, including two serovars carrying the O antigen XV, showed no hemolysis on sheep blood agar. The colonies of the other isolated serovars exhibited varying patterns of hemolysis, i.e., from very pronounced beta-hemolysis to hemolysis that was hardly visible or even completely lacking. Sometimes hemolysis first became visible in subcultures.

Only 37 out of 103 strains tested were virulent for mice, and they were found practically in all areas. Most isolates of serovar 4b, but only two of serovar 1/2b, were virulent (Table 4).

TABLE 3. New O antigen combinations found among the isolated strains

Antigen combination	No. of strains
V, VI, X, XI	8
V, (VI), VII, X, XI, XII, XIII	1
V, VI, VII, X, XI	2
V, VI, VIII, X, XI	3
V, VIII	3
V, VII, XV	2
V, X, XI	1

## DISCUSSION

There is increasing evidence for a high incidence of *Listeria* in plants and soil samples. This agrees with the findings of Welshimer (16) indicating that *L. monocytogenes* has a saprophytic life. The present study confirms that *Listeria* can be isolated frequently from old, faded, or moldy plants. Although it was recovered during all seasons, there was a slight increase during autumn. In this context it may be mentioned that the isolation of *L. monocytogenes* from the above sources was independent of the incidence of listeriosis among domestic animals in the same areas.

The existence of the various serovars with and without hemolysis does not allow any definitive conclusion at this time as to whether or not certain nonhemolytic bio- or serovars are merely saprophytes and perhaps belong to subspecies different from *L. monocytogenes*.

The occurrence of *Listeria* in wildlife feeding grounds and in the feces of wild animals raises the question whether *L. monocytogenes* is an inhabitant of the normal intestine and whether there is a cycle between animals and the soil-plant environment (14), as suggested by Kampelmacher and van Noorle Jansen (7), who stated that *L. monocytogenes* might have a cycle similar to that of *Salmonella*.

It is rather difficult to evaluate the role of birds with respect to the spread of *Listeria* in nature. Birds carry *L. monocytogenes* in the intestinal tract and are thus obviously able to spread it, as is suggested from its isolation from shrub leaves. One may also speculate whether *L. monocytogenes* survives the winter in a manner similar to that of insect-spread enterococci (10).

It seems unlikely that birds and other wild animals are the essential or only source responsible for the distribution of *Listeria* in nature. The high incidence of *Listeria* in plant and soil samples would indicate that, according to the present concept, it is primarily saprophytic. A

TABLE 4. Incidence of virulent (v) and nonvirulent (nv) strains among the isolates of *L. monocytogenes*

Environment	<i>L. monocytogenes</i> serovar									
	1/2a	1/2b	1/2c	4b	4c	4d	4f	4g	4 <sup>a</sup>	5
Cornfields (maize)		1 nv <sup>b</sup>	1 nv	1 v					1 nv	1 v
Grainfields <sup>c</sup>		1 nv		2 v	1 v					
Cultivated fields		1 nv				1 nv				
Uncultivated fields	4 nv	21 nv		8v, 1 nv	1 v				1 v, 5 nv	3 v
Meadows and pastures	1 nv	1 nv		2 v						
Forest		1 v, 5 nv		1 v			1 nv	1 nv	2 v, 1 nv	
Mud				1 v, 1 nv					1 v, 3 nv	
Wildlife feeding grounds	1 v, nv	6 nv		4 v			1 nv		1 nv	
Residues of fodder from wildlife feeding grounds				1 v						
Feces				3 v	1 nv	1 v			1 nv	
Birds	1 v, 1 nv	1 v		1 nv						

<sup>a</sup> New antigen combinations, not yet designated (see Table 3).

<sup>b</sup> Number of strains.

<sup>c</sup> One *L. grayi* (nonvirulent) was isolated from grainfields.

subdivision of *L. monocytogenes* in a hemolytic and a nonhemolytic nonvirulent subspecies may perhaps help to clarify in the future the still obscure pattern of distribution.

Although many questions remain unresolved, at this time the following tentative conclusions are drawn. (i) *L. monocytogenes* and a nonvirulent variety apparently exist as a saprophytic organism in soil and on plants. (ii) *L. monocytogenes* can also be present as an intestinal inhabitant of animals and thus can be spread to the environment. (iii) Humans and animals are exposed to this organism frequently in the natural environment.

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