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Listeria monocytogenes meningitis: serotype distribution and patient characteristics in The Netherlands, 1976–95

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SUMMARY

Two hundred and seven cases of listeria meningitis that occurred in The Netherlands over 20 years were reviewed to study associations between *Listeria monocytogenes* serotype, age, underlying disease, and outcome. The mean annual incidence per 100000 population was 0·12 in 1981–90, decreasing to 0·07 in 1991–5. Underlying disease was present in 50% of non-neonatal patients, most often haematological malignancy (15%) and the use of immunosuppressive therapy (14%). The meningitis-related case fatality rate was 16%; a significantly higher rate was associated with the presence of underlying disease (30%) or age $\geqslant$ 70 years (29%). Serotype 4b was most frequent (65%) and *L. monocytogenes* types 1/2a, 1/2b, or 1/2c (30% of cases) were significantly more often isolated from non-neonatal patients with underlying disease, suggesting a higher virulence of listerial serotype 4b.

INTRODUCTION

Listeria monocytogenes, a Gram-positive bacterium found in a number of ecological sites, causes primarily sepsis and meningitis [1, 2]. The association between contaminated food and both epidemic and sporadic listeriosis has been well documented [3–7]. Meningitis caused by *L. monocytogenes* occurs mainly in the neonatal setting, in the elderly, and in immunocompromised patients [8, 9], although previously healthy adults can be affected as well [10, 11]. Despite the diversity of serotypes of *L. monocytogenes*, only three are responsible for $>$ 90% of human disease: types 1/2a, 1/2b, and 4b [2].

We reviewed 207 cases of meningitis due to *L. monocytogenes* that occurred in The Netherlands over a 20-year period (1976–95). The main purpose of the study was to determine associations between serotype, age, underlying disease that predisposes to listerial infection, and outcome.

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METHODS

All patients from whom a *L. monocytogenes* CSF isolate was submitted from January 1976 to December 1995 to The Netherlands Reference Laboratory for Bacterial Meningitis of the Academic Medical Center and the National Institute of Public Health and the Environment were included in the study. The Reference Laboratory receives 80% of all CSF isolates in The Netherlands (approximately 14·5 million inhabitants in 1985) [12]. In order to calculate a mean annual incidence we therefore multiplied the number of isolates received that year by 1·25. All clinical microbiological laboratories in The Netherlands participate in this surveillance. *L. monocytogenes* was

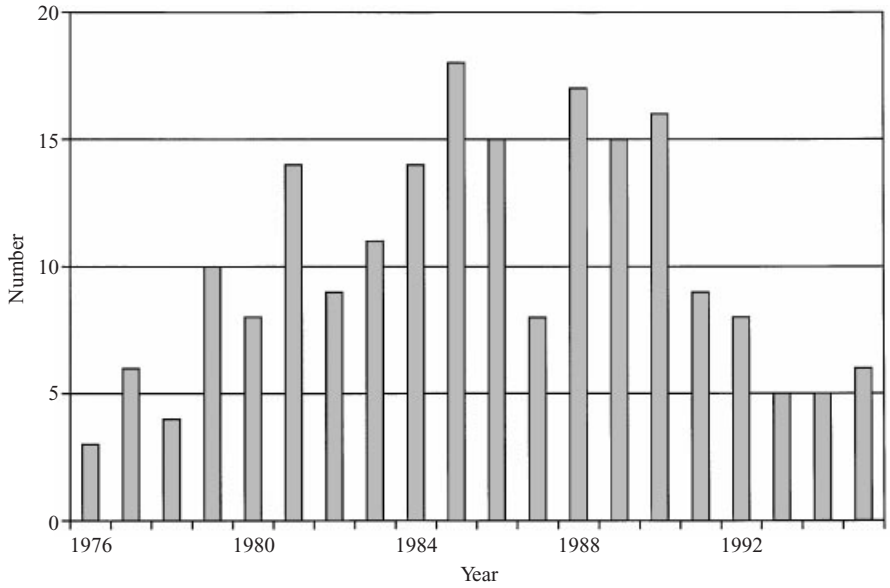

Fig. 1. Annual number of cases of meningitis due to *L. monocytogenes* in The Netherlands, 1976–95.

identified by standard microbiological methods [13]. Serotyping was done as described elsewhere [14].

Information about patients came from medical records and discharge letters that were obtained from hospitals to which the patients had been admitted. We collected data on underlying disease and outcome in terms of case fatality rate and neurological sequelae. Conditions considered to be predisposing to listerial infection included haematological and solid malignancy, connective tissue disease, diabetes mellitus, alcoholism, and the use of immunosuppressive therapy [15].

For computerized data processing, Reflex (Borland, Scotts Valley, CA) was used. Differences of percentages in patient groups were compared with Fisher's exact test or by χ^2 test with Yates' correction and were also expressed as odds ratio with 95% confidence intervals (CI). Log-linear analysis was performed using version 7·5 of the Statistical Package for the Social Sciences (SPSS, Chicago, IL).

RESULTS

Epidemiology. During the study period, the Reference Laboratory received isolates of *L. monocytogenes* from 207 patients with meningitis. A medical record or discharge letter was available for 147 patients (71%). Due to the retrospective nature of the study, the data collected were not complete for every single case. Age and sex were known for 196 (95%) and 204 (99%) patients respectively, while both age and sex were known for 193 patients (93%). Twenty percent of cases occurred in newborns; 12 of the neonatal cases that occurred in the period 1976–82 have been described elsewhere [16]. Thirty-seven percent of the neonatal cases were early-onset infections (< 5 days post-partum). The male/female ratio was 1·6, 61% being male patients.

The annual number of cases of listeria meningitis increased during the first half of the study period, but fewer cases were seen in the 1990s (Fig. 1). On the basis that our data represented 80% of all cases of listeria meningitis in The Netherlands, we calculated mean annual incidences (per 100000 population) of 0·05 in 1976–80, 0·11 in 1981–5, 0·12 in 1986–90, and 0·07 in 1991–5. The date of hospitalization was known for 160 patients. Listeria meningitis was seen significantly more often during the summer months June, July, and August (52 cases [32·5%, 95% CI: 25·4–40·4%]). All cases of listeria meningitis in this series were sporadic; no outbreaks or case clusters were noted.

Underlying conditions. At the time of diagnosis, 64 (50%) of 129 non-neonatal patients had at least one underlying condition, most frequently a haematological malignancy. Eighteen patients (14%) were receiving immunosuppressive therapy. Underlying conditions were present significantly more often in patients aged 40 years or more than in those younger (59/100 [59%] *vs.* 5/29 [17%], $P = 0{\cdot}0002$; odds ratio: 6·9, CI: 2·3–24·8).

Bacteriology. Two hundred and four isolates were serotyped. Of these, 133 (65%) were type 4b and 62

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Table 1. *Distribution of serotypes in 129 non-neonatal patients with meningitis due to* L. monocytogenes*, in relation to underlying disease*

Underlying condition	No. (%) of patients infected with serotype*			
	1/2a, 1/2b, or 1/2c	4b	Other	Total
Underlying condition present	28 (65)	33 (43)	3 (33)	64 (50)
Haematological malignancy	11 (26)	6 (8)	2 (22)	19 (15)
Solid malignancy	6 (14)	3 (4)	0 (0)	9 (7)
Immunosuppressive therapy	8 (19)	8 (10)	2 (22)	18 (14)
Diabetes mellitus	4 (9)	7 (9)	0 (0)	11 (9)
Pregnancy	1 (2)	0 (0)	0 (0)	1 (1)
Alcoholism	2 (5)	6 (8)	0 (0)	8 (6)
Other	2 (5)	14 (18)	0 (0)	16 (12)
No underlying condition	12 (28)	39 (51)	5 (56)	56 (43)
Underlying condition unknown	3 (7)	5 (6)	1 (11)	9 (7)
Total	43 (100)	77 (100)	9 (100)	129 (100)

* Some patients had more than one underlying condition.

(30%) were 1/2a, 1/2b, or 1/2c. The bacteriology results did not differentiate between 1/2a, 1/2b, and 1/2c in 29 cases so the exact frequency of these three serotypes could not be determined. Less common serotypes were the complex of 3a, 3b, and 3c (6 cases), 6a (2 cases) and 4a (1 case). The serotype distributions among neonatal and non-neonatal patients were similar. Table 1 shows the distribution of serotypes in relation to the presence of underlying conditions. The complex of 1/2a, 1/2b, and 1/2c was significantly more often isolated from non-neonatal patients with underlying conditions than from those without such conditions (28/64 [44%] *vs.* 12/56 [21%], $P = 0{\cdot}017$; odds ratio: 2·9, CI: 1·2–6·9). In addition, the median age of patients with meningitis due to serotypes 1/2a, 1/2b, and 1/2c was 57 years compared with 44 years in the group of patients with meningitis due to serotype 4b.

Outcome. The overall case fatality rate was 25% (37/147), with 24 of the 37 deaths (16%) being due to listeria meningitis, and the others related to the underlying disease. In patients younger than 40 years, all fatal cases were meningitis-related. Patients aged 70 years and older died significantly more often than younger ones (10/34 [29%] *vs.* 14/113 [12%]; $P = 0{\cdot}037$; odds ratio: 2·95, CI: 1·06–8·17). A lower case fatality rate was associated with the age group 1 month–39 years (1/29 [3%] *vs.* 23/118 [19%], $P = 0{\cdot}047$, Fisher's exact test; odds ratio: 0·15, CI: 0·0–1·0). Also, the meningitis-related case fatality rate was significantly higher in non-neonatal patients with underlying conditions than in those without such conditions (19/64 [30%] *vs.* 2/56 [4%], $P = 0{\cdot}0004$; odds ratio: 11·4, CI: 2·5–104·4). Gender and serotype were not associated with case fatality rate. In a log-linear analysis the associations between case fatality rate, underlying conditions, age, and serotype were tested. The only significant interactions were those of underlying conditions with case fatality rate ($P = 0{\cdot}0001$), age ($P < 0{\cdot}0001$), and serotype ($P = 0{\cdot}02$).

No recurrent episodes of listeria meningitis were seen. Twenty-two out of 123 surviving patients (18%) developed neurological sequelae. The 16 neonatal survivors were followed for up to 3·5 years (median 11 months). Neurological sequelae developed significantly more often in these neonatal patients than in older patients (7/16 [44%] *vs.* 15/107 [14%], $P = 0{\cdot}009$, Fisher's exact test; odds ratio: 4·8, CI: 1·4–17·0), the most common being hydrocephalus (five cases). Gender, the presence of underlying conditions, and serotype were not associated with the development of sequelae.

DISCUSSION

We reviewed 207 cases of listeria meningitis seen in the Netherlands over a 20-year period. The estimated mean annual incidence over the two decades was 0·07 per 100000. We observed a decline in the incidence of listeria meningitis in the 1990s; this could be accounted for by effective microbiological monitoring of food sources, especially of soft cheeses, for *L. monocytogenes* [17], although a reporting artifact cannot be ruled out. Our data confirm well-established

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epidemiological knowledge about meningitis caused by *L. monocytogenes*. Newborns, the elderly, and immunocompromised patients were the groups at risk. The preponderance of male patients has been observed elsewhere [8, 18–21), as has the seasonal pattern [22]. While pregnant women can account for up to a third of cases of listeriosis [23], this patient group was hardly represented in our series. The rarity of central nervous system infection during pregnancy when other risk factors are absent has been noted [24].

The meningitis-related case fatality rate in this study (16%) is comparable to rates seen elsewhere. Schlech et al. reported a case fatality rate of 28·5% for listeria meningitis in the United States in 1978–81 [25]. In an active surveillance study of bacterial meningitis in the United States in 1986, *L. monocytogenes* had an associated case fatality rate of 22% [26]. In 1995, it was the fourth most common cause of bacterial meningitis in The Netherlands, due to the vaccine-related decline in *Haemophilus influenzae* type b meningitis, with a case fatality rate of 15% [27]. In a large retrospective study of bacterial meningitis in adults, the meningitis-related case fatality rate for *L. monocytogenes* was 21% [28].

Two findings in this study deserve closer attention. Firstly, patients older than 70 years had a higher case fatality rate than younger patients. Although this comes as no surprise, it has not been well documented in previous series. The higher case fatality rate that is seen in this series in patients with underlying disease and in those receiving immunosuppressive therapy has also been noted in other studies [15, 29].

Secondly, while the majority of strains were at serotype 4b, we found that the less common serotypes 1/2a, 1/2b, and 1/2c were more often associated with underlying disease. Also, patients from whom types 1/2a, 1/2b, or 1/2c were isolated were older. These results suggest that serotype 4b of *L. monocytogenes* is more virulent than others. Although experimental data are lacking, some observations support this hypothesis. Strains of *L. monocytogenes* serotype 4b have been predominantly responsible for confirmed outbreaks of food-borne listeriosis [30–32]. In an analysis of the distribution of serovars of *L. monocytogenes* isolated from 1363 patients with listeriosis, serovar 4b occurred more often in previously healthy non-pregnant cases than in those with underlying illness [33]. Following their evaluation of the role of foods in sporadic listeriosis, Pinner et al. suggested that serotype 4b may have an increased capacity to cause disease [34].

Our study provides epidemiological support for a more pronounced virulence of listerial serotype 4b in causing meningitis. Further research into virulence factors of *L. monocytogenes* might refine this observation [35] and elucidate the underlying molecular basis.

REFERENCES

1. Lorber B. Listeriosis. Clin Infect Dis 1997; **24**: 1–11.
2. Gellin BG, Broome CV. Listeriosis. JAMA 1989; **261**: 1313–20.
3. Schlech WF III, Lavigne PM, Bortolussi RA, et al. Epidemic listeriosis – evidence for transmission by food. N Engl J Med 1983; **308**: 203–6.
4. Fleming DW, Cochi SL, Macdonald KL, et al. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. N Engl J Med 1985; **312**: 404–7.
5. Linnan MJ, Mascola L, Lou XD, et al. Epidemic listeriosis associated with Mexican-style cheese. N Engl J Med 1988; **319**: 823–8.
6. Büla CJ, Bille J, Glauser MP. An epidemic of food-borne listeriosis in western Switzerland: description of 57 cases involving adults. Clin Infect Dis 1995; **20**: 66–72.
7. Schuchat A, Deaver KA, Wenger JD, et al. Role of food in sporadic listeriosis. I. Case-control study of dietary risk factors. JAMA 1992; **267**: 2041–5.
8. Kessler SL, Dajani AS. Listeria meningitis in infants and children. Pediatr Infect Dis J 1990; **9**: 61–3.
9. Calder JAM. Listeria meningitis in adults. Lancet 1997; **350**: 307–8.
10. Zúñiga M, Aguado JM, Vada J. *Listeria monocytogenes* in previously healthy adults: long-term follow-up. Q J Med 1992; **85**: 911–5.
11. Hearmon CJ, Ghosh SK. *Listeria monocytogenes* in previously healthy adults: Postgrad Med J 1989; **65**: 74–8.
12. Spanjaard L, Bol P, Ekker W, Zanen HC. The incidence of bacterial meningitis in the Netherlands – a comparison of three registration systems, 1977–1982. J Infect 1985; **11**: 259–68.
13. Swaminathan B, Rocourt J, Bille J. Listeria. In: Murray PR, Baron EJ, Pfaller MA, Tenover MC, Yolken RH, eds. Manual of clinical microbiology, 6th ed. Washington, DC: American Society for Microbiology, 1995: 341–8.
14. Notermans S, Chakraborty T, Leimester-Wächter M, et al. Specific gene probe for detection of biotyped and serotyped Listeria strains. Appl Environ Microbiol 1989; **55**: 902–6.
15. Skogberg K, Syrjänen J, Jahkola M, et al. Clinical presentation and outcome of listeriosis in patients with and without immunosuppressive therapy. Clin Infect Dis 1992; **14**: 815–21.
16. Mulder CJJ, Zanen HC. *Listeria monocytogenes* neonatal meningitis in the Netherlands. Eur J Pediatr 1986; **145**: 60–2.

https://doi.org/10.1017/S0950268802006969 Published online by Cambridge University Press

17. TNO Preventie en Gezondheid. Staat van de Gezondheidsbescherming 1997. Rijswijk: Staatstoezicht op de Volksgezondheid, 1997.
18. Lavetter A, Leedom JM, Mathies AW Jr, Ivler D, Wehrle PF. Meningitis due to *Listeria monocytogenes*: a review of 25 cases. N Engl J Med 1971; **285**: 598–603.
19. Pollock SS, Pollock TM, Harrison MJG. Infection of the central nervous system by *Listeria monocytogenes*: a review of 54 adult and juvenile cases. Q J Med 1984; **53**: 331–40.
20. Hansen PB, Jensen TH, Lykkegaard S, Kristensen HS. *Listeria monocytogenes* meningitis in adults: sixteen consecutive cases, 1973–1982. Scand J Infect Dis 1987; **19**: 55–60.
21. Peeters A, Waer M, Michielsen P, Verbist L, Carton H. *Listeria monocytogenes* meningitis. Clin Neurol Neurosurg 1989; **91**: 29–36.
22. Cherubin CE, Marr JS, Sierra MF, Becker S. Listeria and gram-negative bacillary meningitis in New York City, 1972–1979. Frequent causes of meningitis in adults. Am J Med 1981; **71**: 199–209.
23. McLauchlin J. Human listeriosis in Britain, 1967–1985, a summary of 722 cases. 1. Listeriosis during pregnancy and in the newborn. Epidemiol Infect 1990; **104**: 181–90.
24. Boucher M, Yonekura ML. Listeria meningitis during pregnancy. Am J Perinatol 1984; **1**: 312–8.
25. Schlech WF III, Ward JI, Band JD, Hightower AW, Fraser DW, Broome CV. Bacterial meningitis in the United States, 1978 through 1981. JAMA 1985; **253**: 1749–54.
26. Wenger JD, Hightower AW, Facklam RR, Gaventa S, Broome CV and the Bacterial Meningitis Study Group. Bacterial meningitis in the United States, 1986: report of a multistate surveillance study. J Infect Dis 1990; **162**: 1316–23.
27. Hoogkamp-Korstanje JAA. Infecties door *Listeria monocytogenes*. Ned Tijdschr Geneeskd 1996; **140**: 644–6.
28. Durand KL, Calderwood SB, Weber DJ, et al. Acute bacterial meningitis in adults. A review of 493 episodes. N Engl J Med 1993; **328**: 21–8.
29. Pigrau C, Almirante B, Pahissa A, Gasser I, Vazquez JMM. Clinical presentation and outcome in cases of listeriosis. Clin Infect Dis 1993; **17**: 143–4.
30. Farber JM, Peterkin PI. *Listeria monocytogenes*, a food-borne pathogen. Microbiol Rev 1991; **55**: 476–511.
31. Schuchat A, Swaminathan B, Broome CV. Epidemiology of human listeriosis. Clin Microbiol Rev 1991; **4**: 169–83.
32. Update: multistate outbreak of listeriosis – United States, 1998–1999. MMWR 1999; **47**: 1117–8.
33. McLauchlin J. Distribution of serovars of *Listeria monocytogenes* isolated from different categories of patients with listeriosis. Eur J Clin Microbiol Infect Dis 1990; **9**: 210–3.
34. Pinner RW, Schuchat A, Swaminathan B, et al. Role of foods in sporadic listeriosis. II. Microbiologic and epidemiologic investigation. The Listeria Study Group. JAMA 1992; **267**: 2046–50.
35. Vines A, Swaminathan B. Identification and characterization of nucleotide sequence differences in three virulence-associated genes of *Listeria monocytogenes* strains representing clinically important serotypes. Curr Microbiol 1998; **36**: 309–18.