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Listeria faecal carriage by renal transplant recipients, haemodialysis patients and patients in general practice: its relation to season, drug therapy, foreign travel, animal exposure and diet

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SUMMARY

About 2·3% (16/700) of faecal specimens from renal transplant recipients and patients having home haemodialysis as well as patients attending their general practitioners with symptoms of gastroenteritis yielded *Listeria* species 40% of positive faeces contained more than one *Listeria* species or serovar. The proportion of positive specimens was similar in all three patient groups. Listeria were isolated from 5·6% (10/177) of renal transplant recipients on one or more occasions over the period of a year. The commonest species was *L. monocytogenes* and type 4b the commonest serovar. Carriage was more common in July and August than other times of year, and less than 28 weeks in duration. In renal transplant recipients carriage was positively related to treatment with ranitidine, consumption of more than three types of cheese in the previous 20 months, and consumption of English cheddar cheese more than once per week.

INTRODUCTION

Members of the listeria genus are human and veterinary pathogens and occur widely in the environment and in foodstuffs. Infections may occur in pregnant women and neonates or in the immunosuppressed, particularly in those with malignancies or have had renal transplants [1]. It has been shown recently that infection is seasonal [2]. Contamination of foods, especially soft cheese and pre-prepared salads has been incriminated in a number of large outbreaks of human listeriosis [3, 4]. However, the role of contaminated foodstuffs in sporadic or episodic cases is less clear as only a few such cases have been linked directly to food [5].

Faecal carriage of *Listeria monocytogenes* may be up to 16% in prevalence studies [6–8]. In the present study listeria faecal carriage was followed over a period of 1 year in patients who had had renal transplants and a point prevalence study was undertaken in haemodialysis patients. Faeces specimens sent in by General Practitioners (GPs) for the isolation of enteric pathogens formed the control group. The objective was to establish the faecal carriage rate of listeria in the three groups and to assess any relationship to season, foreign travel, animal exposure or diet.

METHODS

Origin and collection of faeces specimens

Between March 1987 and March 1988 renal transplant recipients attending for out-patient review at Southmead Hospital were sent letters requesting them to bring a specimen of faeces on their next clinic visit. In October 1987 faeces specimens were requested by letter from 115 home haemodialysis patients. Control faecal specimens were submitted by GPs from patients with gastroenteritis during the year of the study. Only specimens which yielded no recognized faecal pathogens from patients who were not known to be taking antibiotics were included.

Bacteriological investigation of faeces

Samples were cultured on the day they were received. The cold enrichment method used was similar to that of Lamont and Postlethwaite [9]. A peat-sized sample of faeces was vortexed vigorously in 10 ml Tryptose phosphate broth (TPB) (Oxoid) and stored at 4 °C. At 4-weekly intervals up to 0·1 ml of each sample was transferred, after vortexing, into 10 ml primary selective medium (TPB containing 3·75% potassium thiocyanate, 0·01 % nalidixic acid and 0·0025 % acriflavine). After incubation at 37 °C for 24 h a loopful was spread in acriflavine/nalidixic acid blood agar (0·004 % nalidixic acid and 0·0025 % acriflavine in 5 % defibrinated blood with Columbia agar) and incubated at 37 °C for 48 h. All colony types were examined and those suspected to be listeria, on the basis of macroscopic appearance, Gram stain and catalase reaction, were subcultured on to blood agar and identified to genus level by API STREP system [10]. Since more than one species or serovar may be carried, five colonies from each enrichment broth were identified and serotyped. These organisms were further investigated for fermentation of D-xylose, L-rhamose and α-methyl-D-mannoside and for accentuation of the haemolytic zone around colonies when plated on sheep blood in the vicinity of *Staphylococcus aureus* and *Rhodococcus equi* (the CAMP test). The species were then classified according to Table 14·12 in *Bergey's Manual of Systemic Bacteriology* [11]. All listeria isolates were serotyped and the species confirmed by Dr J. McLauchlin, Central Public Health Laboratory, Colindale, London. Positive controls were included in each batch of subcultures.

Questionnaire on drug therapy, travel, animal exposure and dietary habits and collection of data on the seasonal incidence of infection in Bristol

A self-administered postal questionnaire enquiring into food consumption, foreign travel, and animal contact since 1 January 1987, was sent to renal transplant and haemodialysis patients found to be faecal carriers of listeria. The questionnaire requested information on travel outside the UK since 1 January 1987, and on ownership and/or contact with cattle, cats, chickens, sheep and goats. Patients were asked which of the following foods they had consumed since 1 January 1987 and if so how often.

Cheese (37 named varieties), yoghurt, unpasteurized cows' milk, goats' milk, sheeps' milk, pre-packed shop-bought vegetables or potato salads, pre-cooked ham, pasties, chicken, meat pies, paté, home-cooked pork, beef, veal, chicken, turkey and lamb. A question on microwave ownership and use was also included. A group of patients who attended the clinic on the same day as the carriers and who were seen immediately before or after the carriers were selected to receive the questionnaire as were the control group consisting of 20 transplant and 4 haemodialysis patients. Information regarding the patient's age, sex, occupation, medical conditions and drug therapy was collected from their medical records.

Data on the isolation of listerias by season in the Bristol area between January 1983 and December 1988 was collected by manual and computer searches of the files in the microbiology laboratories of Southmead and Frenchay Hospitals and the Bristol Royal Infirmary.

Statistical analysis was by χ^2 test with Yates' modification for small numbers.

RESULTS

Occurrence of listeria and related species in faeces

Sixteen of 700 specimens of faeces from patients contained *Listeria* species. Of the 449 specimens obtained from renal transplant recipients, 26 were from in-patients shortly after surgery and 423 were from out-patients. Of the 177 renal transplant recipients who submitted these specimens, 58 submitted 1, 49 submitted 2, 30 – 3 specimens, 17 – 4 specimens, 12 – 5 specimens, 5 – 6 specimens, 3 – 7 specimens and 3 – 8 specimens, all over the year of study. The carriage rate was 5·6% (10/177) over the period of a year and 2·5% (11/449) specimens contained listeria.

One hundred and seventy-one specimens were collected from patients attending GPs over a year and 80 from haemodialysis patients in October 1987. As far as is known each patient submitted a single specimen only. 1·8% (3/171) of samples from general practice patients and 2·5% (2/80) from home haemodialysis patients were positive. The proportion of positive specimens from the pooled data of all three groups varied throughout the year (Table 1), being lowest between September and February (range 0·8–1·6%) and highest in July and August (5·2%). Most of the isolates ($n = 7$) were recovered from faeces in July or August and the peak incidence of listeriosis in Bristol was 2 months later in September and October when 8/26 (30%) of the clinically and microbiologically documented infections occurred (Table 2).

The identity of the isolates and their serotypes are shown on Table 3. Ten transplant patients were faecal carriers, one patient had two different isolates in consecutive specimens, and mixed *Listeria* sp. were isolated from single faeces from four patients. One patient was excreting two different serovars of *L. monocytogenes* and a single strain of *L. innocua* was also present. All the patients from whom listeria was isolated submitted between 3–6 specimens in the course of the year. A total of 21 strains of listeria were isolated from all the patients examined; most were *L. monocytogenes* (14/21) 6 of which were serovar 1/2 and 8 serovar 4. Six *L. innocua* and one *L. welshimeri* were isolated.

The period of carriage in the five transplant patients who submitted multiple samples was between 1 and 28 weeks.

Table 1. Listeria *faecal carriage in renal transplant recipients, haemodialysis patients and patients in general practice and its seasonal variation between March 1987 and 1988*

Month	Renal transplant		General Practice		Haemodialysis		All groups	
	Number faeces examined	Positive	Number faeces examined	Positive	Number faeces examined	Positive	Number faeces examined	Positive
Jan., Feb. 1988	61	1	15	0	0	0	76	1 (1·3%)
Mar. 1987/8, Apr. 1987	86	1	7	0	0	0	93	2 (2·1%)
May, June 1987	68	1	23	1	0	0	91	2 (2·2%)
July, Aug. 1987	75	5	60	2	0	0	135	7 (5·2%)
Sept., Oct. 1987	74	1	32	0	78	2	184	3 (1·6%)
Nov., Dec. 1987	85	1	34	0	2	0	121	1 (0·8%)
Total	449	11 (2·5%)	171	3 (1·8%)	80	2 (2·5%)	700	16 (2·3%)

Table 2. *The number of listeria infections in Bristol and asymptomatic faecal isolation occurring through the year*

Months	Faecal isolates ($n = 16$)	Infections ($n = 26$)
January, February	1 (6%)	4 (15%)
March, April	2 (12%)	3 (11%)
May, June	2 (12%)	0 (0%)
July, August	7 (44%)	6 (23%)
September, October,	3 (19%)	8 (30%)
November, December	1 (6%)	5 (19%)

Table 3. *Patient details and* Listeria *species cultured from faecal carriers*

Patient	Date specimen submitted	Group	Species	Serovar	Number of specimens submitted by patient	Number of isolates identified
1	17 Mar. 88	Transplant	*monocytogenes*	1/2	3	6
2	7 Apr. 87	Transplant	*monocytogenes*	1/2	4	6
3	5 May 87	Transplant	*monocytogenes*	4b	3	6
			innocua	nk		
4	7 July 87	Transplant	*monocytogenes*	4b	4	6
5	5 May 87	Transplant	*monocytogenes*	4b	4	8
			innocua	nk		
	25 Aug. 87		*welshimeri*	nk	4	6
6	11 Aug. 87	Transplant	*monocytogenes*	4b	4	6
7	25 Aug. 87	Transplant	*monocytogenes*	1/2	4	6
			innocua	nk		
8	13 Oct. 87	Transplant	*monocytogenes*	4b	4	11
			monocytogenes	1/2		
			innocua	nk		
9	7 Dec. 87	Transplant	*monocytogenes*	1/2	6	6
10	29 Feb. 88	Transplant	*innocua*	nk	3	6
11	30 June 87	Gp practice	*monocytogenes*	4b	1	6
12	18 Aug. 87	Gp practice	*monocytogenes*	4b	1	6
13	18 Aug. 87	Gp practice	*monocytogenes*	1/2	1	6
14	14 Oct. 87	Haemodialysis	*monocytogenes*	4	1	3
15	15 Oct. 87	Haemodialysis	*monocytogenes*	4	1	3

nk, not known.

Listeria questionnaire results

The results of the listeria questionnaire are presented in Tables 4–6. The medical records were available for 8/10 transplant patients and both the haemodialysis patients who were faecal carriers. Amongst the control group the notes were available for all 20 transplant and 2 of the 4 haemodialysis patients. Nine (75%) of the listeria carriers returned the questionnaires (8 transplant and 1 haemodialysis patient) while 19 controls (15 transplants and 4 dialysis patients) responded.

The listeria carriers and control groups were well matched for age, sex and

Table 4. *Age, sex, underlying renal and medical conditions and drug therapy of renal transplant recipients*

	Listeria carriers ($n = 10$)	Control group ($n = 22$)	Significance
Age (years)	43 ± 10*	46 ± 14	n.s.
Sex (male/female)	9/1	16/6	
Number of transplant recipients	8	20	
Cause of renal failure			
Glomerulonephritis	5	5	
Polycystic disease	0	3	
Other	4	9	
Length of time post transplant specimen submitted (months)	30 ± 42	52 ± 27	n.s.
Concurrent medical conditions			
Diabetes melitus	0	2	n.s.
Neoplasia	0	1	n.s.
Upper gastro-intestinal disease	3	3	n.s.
Drug therapy			
Prednisolone (mg/day)	7·5 ± 3·5	7·3 ± 1·5	n.s.
Azothioprine (mg/day)	110 ± 53	129 ± 38	n.s.
Number of patients receiving:			
Cyclosporin A	1	4	n.s.
Rantidine	4	0	$P < 0·01$
Antibiotics	0	0	n.s.

* mean ± standard deviation.

Table 5. *Number of carriers and controls eating specified cheeses since January 1987*

Cheese	Carrier ($n = 8$)	Controls ($n = 19$)	Significance
Stilton	5	7	n.s.
Brie	4	4	n.s.
Irish Cheddar	3	3	n.s.
English Cheddar	8	17	n.s.
New Zealand Cheddar	3	3	n.s.
Bel Paese	2	0	n.s.
Cheshire	3	1	n.s.
Percentage eating 3 or more types of cheese	6	6	$P < 0·1$

n.s., not significant.

underlying renal and medical conditions (Table 4). Patients who were carriers had been transplanted more recently than the control group but this difference was not significant. There were no significant differences in immunosuppressive therapy but significantly more listeria carriers were receiving the H_2-antagonist, ranitidine, than the control group ($P < 0·01$).

There was no significant association between listeria carriage and travel abroad with 33% of carriers with 21% of controls travelling outside the UK since January 1987. No respondents had been in physical contact with farm animals while 4/9 of carriers of 9/18 of controls had touched or stroked a cat in the

Table 6. *Frequency of pre-cooked and home-cooked meat consumption*

	Number eating different meats more often than once/month		
	Carriers	Controls	Significance
Pre-cooked			
Ham	4/9*	5/18*	n.s.
Pastie	0/8	3/13	n.s.
Chicken	0/7	3/14	n.s.
Meat pie	0/8	3/15	n.s.
Paté	1/8	1/18	n.s.
Home cooked			
Pork	7/9	2/17	n.s.
Beef	6/9	10/18	n.s.
Veal	0/9		n.s.
Chicken	9/9	14/7	n.s.
Turkey	1/9		n.s.
Lamb	5/9	10/18	n.s.

n.s., not significant.
* Denominators vary as not all patients answered unambiguously.

previous 20 months. Cats were owned by 4 people in the carrier group and 7 in the control group. No other pets were owned.

Listeria faecal carriers ate significantly more types of cheese when compared to controls: 6/8 of listeria carriers listed 3 or more cheeses as being eaten since Jan 1987 compared to 6/18 in the control group (Table 5). More detailed questions on the type of cheese eaten revealed that significantly more carriers (8/8) consumed English cheddar, at least once per week, than controls (5/19) ($P < 0{\cdot}05$).

There was no significant difference in the consumption of other cheeses, yoghurts, pre-cooked or home-cooked meats between the control group and faecal carriers (Table 6). Eight of 9 listeria carriers owned or used a microwave for cooking compared to 9 of 16 in the control group, a difference that was not significant.

DISCUSSION

Listeria faecal carriage rates are likely to be greatest where multiple specimens are provided. In our two groups in which subjects provided single specimens, the general practice and home haemodialysis patients, the carriage rates were similar at 1·8 and 2·5% respectively. These figures are slightly lower than in two other faecal carriage studies in the UK where carriage rates were 4·0 and 5·2% [9, 12]. Higher carrier rates have been reported in the Netherlands and Germany [12]. In the Netherlands, when multiple samples were taken, a carriage rate of 67% was found over an 8-week period in laboratory workers [13]. The listeria faecal carriage rate of renal transplant recipients is no different from that in other patients. Similarly pregnant women, who are also in a high-risk group, do not have increased faecal carriage [9].

The apparent seasonal variation in human carriage has not been described previously although higher counts in sewage effluent have been recorded in

September than in January [14] and soil samples from fields where listeria was isolated in the autumn were negative in the spring [15]. The peak incidence of infection in Bristol is in September and October which is approximately 8 weeks after the peak in faecal carriage. Epidemiological studies of listeria outbreaks indicate that the incubation period of listeriosis is 30–35 days which would be compatible with this time difference.

It is known that more than one species or serovar of listeria can be isolated from the same sample of human faeces [13] and in 40% of faecal carriers in this study more than one strain of listeria was found. Our most commonly identified serovar is 4b which is in contrast to the findings of Kampelmacher and van Noorle Janset in the Netherlands [13] and Lamont and Postlethwaite in Scotland [19] who found their commonest serotypes to be 1/2 and 3, respectively. *L. innocua* is a common faecal isolate [19] but *L. welshimeri* is not. Only 2 of 28 strains of *L. welshimeri* identified by Rocourt Seeliger [16] were isolated in Europe and the usual site of isolation is environmental rather than man or animals.

Epidemiological studies of over 50 sporadic cases of infection have failed to establish a link between infection and previous antibiotic or immunosuppressive therapy [17]. Which is similar to our data on faecal carriage. However, Stamm and colleagues [18] showed that in renal transplant recipients listeria infection often occurred when the dose of daily prednisolone was > 30 mg and was rare of the dose was < 15 mg/day. None of our renal transplant patients who were listeria faecal carriers was receiving more than 15 mg/day of prednisolone, and no cases of clinical infection occurred in any faecal carriers.

Both steroids and azothioprine reduce immunity to listeria in mice, but this may only be of importance in man when higher doses are used to suppress rejection episodes rather than during maintenance treatment [19, 20].

Studies on the association between H_2-antagonists and listeria infection have been conflicting. Some support the relationship, the others do not [17, 21]. The present study provides evidence that H_2-antagonists may be associated with listeria faecal carriage. An analogous situation exists for another food-borne pathogen, salmonella [22].

The only food association with faecal carriage was with the number of different types of cheese each individual had eaten. That the carriers ate English Cheddar is probably coincidental as listeria is not found in such hard cheeses [23]. The association between cheese consumption and faecal carriage may be more marked than with meats because the level of listeria contamination is generally 1000-fold higher in cheese than in meat and the numbers of organisms tend to decrease with time in meat while they tend to increase in cheese [24]. Therefore, the inocula in contaminated cheeses may tend to be larger and might more readily establish faecal carriage, which has been shown to be inhibited by endogenous bowel flora in mice [25].

In conclusion, listeria faecal carriage in renal transplant, haemodialysis patients and those in general practice were not markedly different. Carriage was seasonal, being more common in July and August and in transplant recipients related to ranitidine therapy and cheese consumption but not immunosuppressives. No renal transplant patients who were carriers developed infection but all were only on low doses of prednisolone used for maintenance immunosuppression.

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REFERENCES

1. Lamont RJ, Postlethwaite R, MacGowan AP. *Listeria monocytogenes* and its role in human infection. J Infect 1988; **17**: 7–28.
2. McLauchlin J. Listeriosis in Britain 1967–85, a summary of 722 cases of listeriosis in non pregnant individuals, a changing pattern of infection and seasonal incidence. Epidemiol Infect 1990; **104**: 191–201.
3. Linnan MJ, Mascola L, Dong Lau X, et al. Epidemic listeriosis associated with Mexican-style cheese. N Eng J Med 1988; **312**: 404–7.
4. Pini PN, Gilbert RJ. The occurrence in the UK of *Listeria* species in raw chicken and soft cheeses. Int J Food Microbiol 1988; **6**: 317–26.
5. Bannister BA. *Listeria monocytogenes* meningitis associated with eating soft cheese. J Infect 1987; **15**: 165–8.
6. Borjsen-Möller J. Human listeriosis: diagnosis epidemiology and clinical studies. Acta Pathol Microbiol Scand (Suppl) 1972; **229**: 1–157.
7. Kampelmacher EH, Huysinga WT, van Noorle Jansen LM. The presence of *Listeria monocytogenes* in faeces of pregnant women and neonates. Zentrabl Backteriol Mikrobiol Hyg 1972; **222**: 258–62.
8. Ralovich B. Listeriosis research: present situation and perspective. Akademac Kiodo, Budapest, 1984.
9. Lamont RJ, Postlethwaite R. Carriage of *Listeria monocytogenes* and related species in pregnant and non-pregnant women in Aberdeen, Scotland. J Infect 1986; **13**: 187–93.
10. MacGowan AP, Marshall RJ, Reeves DS. Evaluation of API 20 STREP system for identifying *Listeria* species. J Clin Path 1989; **42**: 548–50.
11. Seeliger HPR, Jones D. Listeria. In Bergey's manual of systematic bacteriology, Mair NS, Sharpe ME, Holt JG. eds. Baltimore; Williams and Wilkins 1986; 1235–45.
12. Kampelmacher EH, van Noorle Jansen LM. Listeriosis in humans and animals in the Netherlands (1958–1877). Zentrabl Bakteriol Mikrobiol 1980; **246**: 211–27.
13. Kampelmacher EH, van Noorle Jansen LM. Further studies on the isolation of *Listeria monocytogenes* in clinically healthy individuals. Zentrabl Backeriol Mikrobiol Hyg 1972; **222**: 258–62.
14. Watkins J, Sleath K. Isolation and enumeration of *Listeria monocytogenes* from sewage, sewage sludge and river water. J Appl Bacteriol 1981; **50**: 1–9.
15. Weis J, Seeliger HPR. Incidence of *Listeria monocytogenes* in nature. Appl Microbiol 1975; **30**: 29–32.
16. Rocourt J, Seeliger HPR. Distribution de espèces du genre *Listeria*. Zentrabl Backteriol Mikrobiol 1985; **259**: 317–30.
17. Schwartz B, Ciesielski CA, Broome CV, et al. Association of sporadic listeriosis with consumption of uncooked hot-dogs and undercooked chicken. Lancet 1988; ii: 779–82.
18. Stamm AM, Dismukes WE, Simmon BP, Glenn Cobhi C, Elliott A, Budrich P, Marmon J. Listeriosis in renal transplant recipients: Report of an outbreak and review of 102 cases. Rev Infect Dis 1982; **4**: 665–82.
19. Miller JK, Hedberg M. Effects of cortisone on susceptibility of mice to *Listeria monocytogenes*, Am J Clin Path 1965; **43**: 248–50.
20. Tripathy SP, MacKaness GB. The effect of cytotoxic agents on the primary immune response to *Listeria monocytogenes*. J Exp Med 1969; **130**: 1–16.

21. Ho JL, Shands KN, Friedland G, Eckind P, Fraser DW. An outbreak of type 4b *Listeria monocytogenes* infection involving patients from eight Boston hospitals. Arch Intern Med 1986, **146**: 520–24.
22. Gionnella RA, Broitman SA, Zamchesk N. Salmonella enteritis: role of reduced gastric secretions in pathogenesis. Am J Dig Dis 1971; **16**: 1009–13.
23. Sizmur K, Walker CW. *Listeria* and food. Lancet 1988; ii: 1167.
24. Breer C, Schopfer K. *Listeria* and food. Lancet 1988; ii: 1022.
25. MacDonald IT, Carter BP. Cell mediated immunity to intestinal infection. Infect Immun 1980; **28**: 516–28.