

RESEARCH ARTICLE

# Risk Factors for Sporadic Non-Pregnancy Associated Listeriosis in Germany—Immunocompromised Patients and Frequently Consumed Ready-To-Eat Products

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**Data Availability Statement:** The data set underlying the findings is available from the Zenodo repository (<https://zenodo.org/record/32864>; DOI [10.5281/zenodo.32864](https://doi.org/10.5281/zenodo.32864)). Patient privacy concerns make raw study data legally and ethically unsuitable for public posting. To ensure anonymity of the patients, age classes and underlying conditions were provided as composite variables.

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## Abstract

Non-pregnancy associated (N-PA) listeriosis, caused by *Listeria monocytogenes*, is a rare but severe disease, and is predominantly food-borne. Most cases appear sporadic and their infection vehicle remains unknown. Incidence has increased since 2008 in Germany. We aimed to identify underlying conditions and foods associated with sporadic N-PA listeriosis in Germany. We performed a nationwide case-control study from March 2012–December 2013. Cases were sporadic N-PA listeriosis patients notified to public health. Control subjects were age (40–65 years, 66–75 years,  $\geq 76$  years) frequency-matched persons from a nationwide random telephone sample. A structured questionnaire collected information on underlying diseases, therapies and >60 food items. We conducted multivariable logistic regression analysis, adjusting for host factors identified by causal diagram theory, and calculated population attributable fractions. We enrolled 109 cases and 1982 controls. Cases' median age was 69 years, 55% were male, 44% received immunosuppressive therapy within 3 months prior to illness onset; a further 28% had at least one immunocompromising disease. In multivariable analysis, immunosuppressive therapy (OR 8.8, 95%CI 4.9–15.6), immunocompromising disease (OR 2.7; 95%CI 1.4–5.2), gastric acid suppression (OR 3.0; 95%CI 1.4–6.3), the consumption of cold cooked sausages (OR 2.6; 95%CI 1.6–4.4), the preferred consumption of packaged cheese (OR 2.1; 95%CI 1.3–3.5) and pre-sliced cheese (OR 2.2; 95%CI 1.3–3.7) were significantly associated with N-PA listeriosis. These foods accounted for 59% of all cases. Typical high risk foods, e.g. cold seafood, certain types of cheeses, tended to be negatively associated with disease. In conclusion, immunosuppressive therapy and frequently consumed ready-to-eat foods are the main risk factors for sporadic N-PA listeriosis in Germany. To reduce their risk, immunocompromised persons should consume the identified foods well before the 'use-by' date. The microbiological criteria for *Listeria monocytogenes* in ready-to-eat foods may insufficiently protect persons who are markedly immunocompromised.

**Competing Interests:** The authors have declared that no competing interests exist.

## Introduction

Listeriosis is a rare but life-threatening disease with high mortality, caused by the bacterium *Listeria monocytogenes*. Host factors relating to reduced immunocompetency are known to play an important role in listeriosis. Cases occur predominantly in the elderly and in immunocompromised persons (non-pregnancy associated, 'N-PA'), and in pregnant women. Moreover, gastric acid suppression increases susceptibility to bacterial enteric infection [1], but an association with listeriosis independent of underlying diseases has not been demonstrated thus far. In adults, common manifestations of listeriosis are meningococcal meningitis, sepsis and focal infections in various organ systems.

*L. monocytogenes* is transmitted predominantly through contaminated food [2]. Various food vehicles have been identified in outbreaks [3–5] including ready-to-eat meat products (e.g. frankfurters), pasteurized and unpasteurized cheese (e.g. soft cheeses), fruit, vegetables and fish products. Case-control studies of risk factors for sporadic illness have produced highly inconsistent results. One failed to identify food items at all [6], others found statistical associations with different foods that could often explain only a minority of the cases [7–9], including hummus and ice milk [9], ready-to-eat beef, smoked fish, prawns, milk, butter, cheese and mixed salads [7, 10]. The importance of these foods or other risk factors for sporadic N-PA listeriosis in Germany has not been systematically investigated so far.

In Germany, detection of *L. monocytogenes* in cultures from a normally sterile site, usually from blood or cerebrospinal fluid, is notifiable to the local Public Health department. Most notified cases appear sporadic, are N-PA (>90%), and the source of infection remains unknown. Listeriosis is associated with the highest mortality (7–9% of notified cases, 2009–2013) and years of potential life lost among classical notifiable enteric pathogens [11], even when neglecting the contribution of pregnancy associated cases. Annual incidence of notified N-PA listeriosis has increased from 0.3 per 100 000 population in 2008 to 0.5 per 100 000 population in 2013. We aimed to identify underlying conditions and foods associated with N-PA listeriosis.

## Methods

### Study Design and Data Collection

We conducted an age frequency-matched case-control study in all German Federal States, except for the Federal city state of Bremen (0.8% of the German population), and enrolled cases prospectively from 15 March 2012 (Baden-Württemberg; 29 May 2012) to 31 December 2013. A case was defined as clinically compatible illness (fever, septicaemia, meningitis or localized organ infection) in a non-pregnant person with isolation of *L. monocytogenes* from a normally sterile site who was notified to a local health department during the study period, but not part of a recognized outbreak for which a common vehicle of infection was identified.

Staff from local health authorities contacted case patients to request informed verbal consent and documented patients' response. Obtaining written informed consent was deemed to be associated with substantial time delay, thereby reducing patients' food history recall, particularly as most patients were still in hospital when contacted by telephone. Consent procedure was approved by the ethics committee.

Case interviews were conducted by staff from the local health authorities or the Robert Koch Institute (RKI). If a case patient was too ill or deceased, proxy interviews with persons familiar with the patient's food history or dietary habits were attempted.

Population-based control subjects were nationwide identified by random sampling of telephone numbers continuously between March 2012 and October 2013. The sample was generated by the GESIS Leibniz Institute for Social Sciences, Mannheim according to the method by

Gabler and Häder [12], taking into account telephone numbers not registered in telephone books. Computer-assisted telephone interviews (CATI) were conducted by the RKI targeting the oldest member of the household. To ensure balanced completion of control-strata, enrollment was split in blocks of 35 persons, i.e. recruitment was interrupted in a stratum after enrollment of 35 participants until the other strata had reached an equal number of enrolled control-subjects. Eligible control subjects had no known history of listeriosis and had to be accessible by telephone in Germany. Control subjects were frequency-matched by age-categories (40–65 years, 66–75 years, ≥76 years), which represented the terciles of the age distribution of reported N-PA-cases between 2004 and 2011. We attempted to recruit four controls per case (n = 400) to be able to identify associations with an odds ratio (OR) of 1.45 as statistically significant (1-β = 0.9; α = 0.05).

The structured questionnaire collected information on socio-demographic characteristics, symptoms of illness (only for cases), knowledge of the disease and transmission routes for listeriosis, underlying diseases, medication, and >60 items of food consumption and purchase during the 4-weeks prior to symptom onset (for cases) or prior to the interview (for controls). Information on underlying conditions was elicited for a) diseases accompanied with reduced immunocompetency and b) immunosuppressive therapies in the 3 months prior to illness onset (cases) or to interview (controls). For a list of conditions see [Table 1](#).

**Table 1. Association\* of underlying conditions with non-pregnancy associated listeriosis in a case control study in Germany, 2012–2013.**

	Cases exposed		Controls exposed		OR*	95% CI	p-value
	n	%	n	%			
<b>Conditions associated with N-PA listeriosis</b>							
<i>immunosuppressive therapy<sup>a</sup></i>							
Chemotherapy	14/108	13.0	17/1976	0.9	17.12	8.17–35.78	<0.001
Immunosuppressive medication	40/98	40.8	189/1960	9.6	6.52	4.23–10.04	<0.001
Radiation therapy	5/108	4.6	17/1978	0.9	5.77	2.08–16.03	0.001
Hemodialysis	4/108	3.7	14/1901	0.7	5.44	1.75–16.96	0.003
<i>immunocompromising disease</i>							
Cancer–hemotological <sup>b</sup>	9/109	8.3	12/1978	0.6	15.97	6.51–39.16	<0.001
Splenectomy	5/106	4.7	9/1975	0.5	10.44	3.42–31.85	<0.001
Liver disease	13/106	12.3	40/1973	2.0	7.14	3.67–13.89	<0.001
Renal disease	17/108	15.7	86/1971	4.4	4.62	2.60–8.22	<0.001
Solid organ transplantation	4/107	3.7	22/1977	1.1	3.89	1.30–11.62	0.015
Autoimmune disorder	9/102	8.8	50/1934	2.6	3.53	1.68–7.41	0.001
Diabetes	29/107	27.1	306/1976	15.5	2.19	1.40–3.45	0.001
<i>Non-immunological conditions</i>							
Gastric acid suppression <sup>a</sup>	17/103	16.5	98/1963	5.0	3.84	2.19–6.72	<0.001
<b>Conditions without association to N-PA listeriosis</b>							
Hypertony	56/106	52.8	999/1972	50.7	1.18	0.79–1.77	0.426
Chronic enteritis	6/107	5.6	74/1965	3.8	1.52	0.65–3.58	0.338
Arthritis	18/99	18.2	289/1954	14.8	1.37	0.81–2.34	0.243
Cancer—solid (last 5 years)	16/106	15.1	208/1973	10.5	1.63	0.93–2.84	0.088
<b>No precondition</b>	11/78	14.1	592/1783	33.2	0.28	0.14–0.54	<0.001

\* single variable analysis, adjusted for age

<sup>a</sup> within the last 3 months

<sup>b</sup> within the last 5 years

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Data from case patients was entered into an EpiData database (version 3.1, The EpiData Association, Denmark) and validated by double data entry. Data from controls were extracted from the CATI database (Voxco, Montreal, Canada) and merged with case data.

## Data Analysis

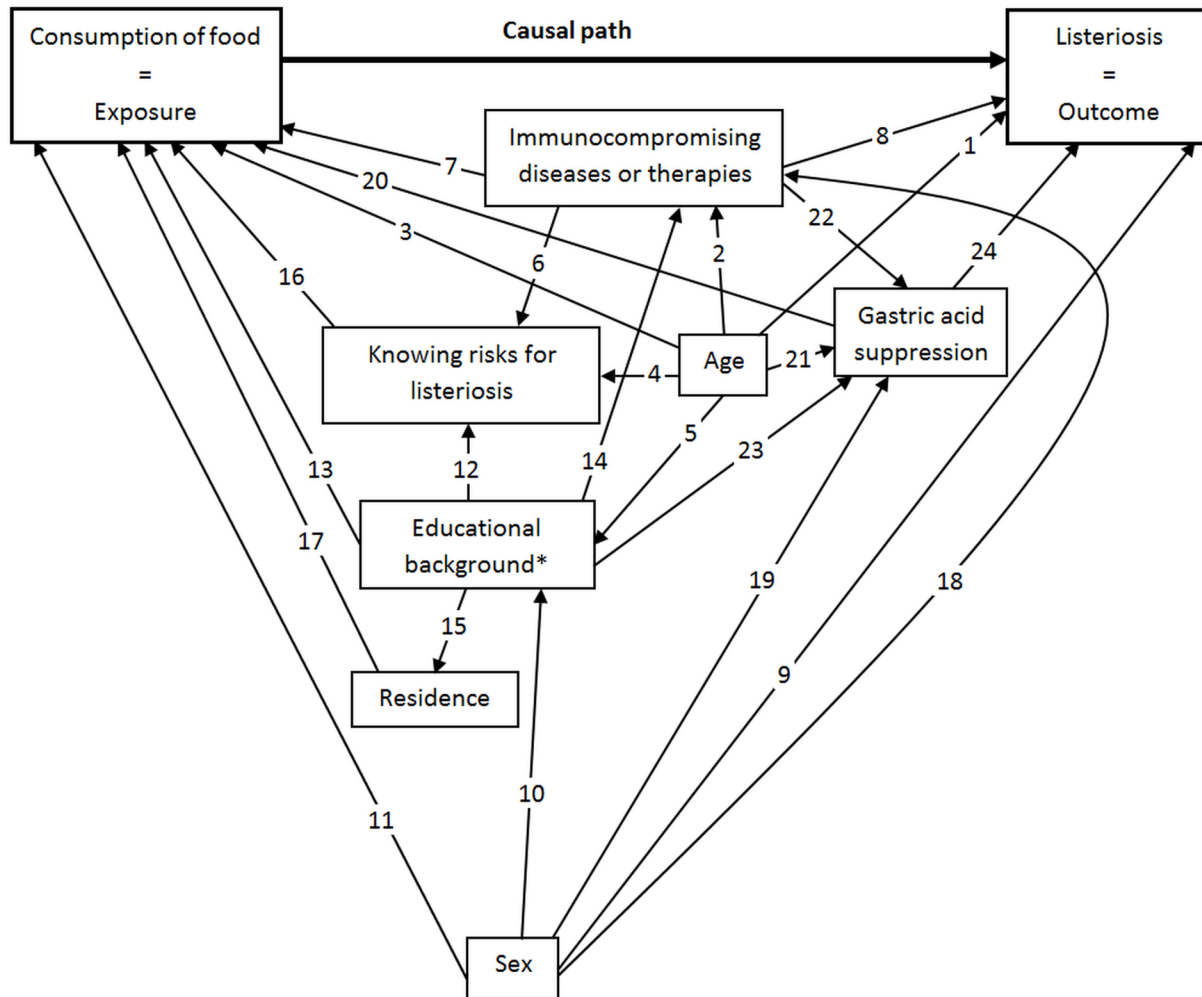
Information for the person's highest educational background was classified as "high" (polytechnic or university degree), "medium" (apprenticeship or [advanced] vocational education), or "low" (no or current training) and used as an indicator for socioeconomic status (SES) [13]. Type of residence was classified as "village" (<2000 inhabitants), "town" (2000–100 000 inhabitants) or "metropolis" (>100 000 inhabitants), according to the definition of Germany's Federal Statistical Office. Food exposure data, which were collected in four frequency categories (from "never" to "daily") were dichotomized. Additionally, we combined various types of cheese that had a negative association with N-PA listeriosis ( $p < 0.05$ ) into a single variable that represented the consumption of at least one negatively associated type of cheese.

The case population was described with respect to frequencies of socio-demographic characteristics, underlying conditions and main disease outcomes.

Cases with information elicited from proxies were excluded from analysis of previous knowledge about listeriosis. Furthermore, knowledge about listeriosis and disease transmission in the study population was investigated with respect to underlying conditions.

Univariable analysis was performed for host variables and for all food related exposure variables. We computed univariable OR and 95% confidence intervals (95% CI) using logistic regression, adjusted for the matching factor age-category. Underlying conditions significantly associated with N-PA listeriosis and known to be associated with reduced immunocompetency [14–18] were grouped into "immunosuppressive therapy in the 3 months prior to illness onset (case) or interview (controls)", e.g. chemotherapy, and "immunocompromising diseases" without immunosuppressive therapy, e.g. diabetes. These categories were mutually exclusive and were compared against "no relevant condition", i.e. none of the conditions included in the former categories (see Table 1). Gastric acid suppression was considered as separate host factor, since we assumed that gastric acid suppression increases susceptibility to enteric infections primarily by non-immunologic mechanisms, thus distancing it from the mechanisms of immunocompromising diseases and therapies.

Multivariable logistic regression modeling was used with the primary objective of investigating the independent relationship of single food-exposures and N-PA listeriosis, while adjusting for the potential confounding effect of host factors. Thereafter, we applied a two step strategy. First, we constructed directed acyclic graphs ('causal diagrams') to visualize the hypothesized causal structure of host factors and N-PA listeriosis. The relationships displayed in the graph were based on statistical associations identified in epidemiological, clinical and nutritional studies, official statistics, or, if the former was lacking, plausible assumptions (Fig 1). Because analytical adjustment for potential confounding variables can create bias where none exist [19], we deduced the minimally sufficient adjustment set to minimize bias using the graphical interface DAGitty [20]. Second, we then employed a manual forward stepwise selection strategy with the forced-in variables of the minimally sufficient adjustment set and food-items as candidate variables that had a  $p$ -value  $< 0.05$  in univariable analysis. Variables for points of food purchase and supply were not considered for multivariable modelling because of too many missing values. We set the  $p$ -value for candidate variables eligible for multivariable modeling narrowly to  $p < 0.05$  to reduce the risk of increasing bias and model non-convergence due to too few outcome events per explanatory variable [21]. Main-effects of the final multivariable logistic regression model were investigated for statistical interactions using the likelihood-ratio test. Model specification was assessed with the link test.



**Fig 1. Hypothesized causal structure of exposure, host factors and non-pregnancy associated listeriosis, Germany, 2012–2013 [48–61].**

\* Proxy for socio-economic status

**Presumed causal relationships based on:**

Significant associations in scientific studies

- 1 Age-related changes of the immune system (immunosenescence) lead to increased susceptibility to infections [48] in general; risk for listeriosis increases with age [49]
- 2 Prevalence of chronic health conditions in Germany increases with age [50]
- 3 Dietary behavior of German adults depends on age [51, 52]
- 5 Socioeconomic status depends on age in a highly representative sample of the German population [13]
- 8 Presence of an underlying immunocompromising disease or therapy increases risk for listeriosis [24, 25]
- 9 Experimental evidence suggests increased susceptibility for listeriosis in male animals [53]
- 10 Socioeconomic status depends on sex in a highly representative sample of the German population [13]
- 11 Dietary patterns among German adults differ between the sexes [51]
- 13 Socioeconomic status influences nutrition pattern [54]
- 14 Health (morbidity) is related to socioeconomic status in highly representative samples of the German population [55]
- 18 Morbidity is related to sex in highly representative samples of the German population [50]
- 19 Prescription of gastric acid suppressors is related to sex in Germany [56]
- 21 Prescription of gastric acid suppressors is related to age in Germany [56]

23 Prevalence of reflux symptoms (indication for gastric acid suppression) is significantly associated to socioeconomic status in a highly representative sample of the German population [57]; chronic use of gastric acid suppressors is associated with low income and low educational level [58]

24 Gastric acid suppression lowered infectious dose of *L. monocytogenes* in animals [59] and was associated with listeriosis outbreak [60]

#### Official Statistics:

17 Residence determines access to retailers, food handlers, farmer's markets etc. ([www.lbv.brandenburg.de/dateien/stadt\\_wohnen/rb\\_Einzelhandel\\_Brandenburg\\_2011.pdf](http://www.lbv.brandenburg.de/dateien/stadt_wohnen/rb_Einzelhandel_Brandenburg_2011.pdf); last accessed on 18.12.2014)

#### Assumptions:

4 Knowledge about listeriosis depends on age (e.g. by lifelong learning, age-dependent access to electronic media)

6 People with underlying disease are better informed about listeriosis through physicians or self-study

7 Consumption pattern of people with underlying immunocompromising diseases or therapy differ from those of healthy people (food intolerances, special diets etc.)

12 People with higher SES (educational background) are better informed about listeriosis

15 SES influences choice of residence

16 Susceptible persons who know listeriosis tend to avoid consumption of risk foods

20 Consumption pattern of people with gastric acid suppression differ from those of people without (weight loss diet is recommended as non-drug treatment for reflux diseases in German treatment guidelines)

22 High level of (inappropriate) prescriptions of gastric acid suppressors for hospitalized patients are continued in primary care [61]

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Population attributable fractions for risk factors in the final multivariable model were estimated with 95% CI based on the method described by Greenland and Drescher [22, 23].

All statistical analyses were performed using Stata/IC 12.1 (StataCorp LP, USA).

## Ethics Statement

Ethics approval was obtained from the Ethics committee of the Charité, Berlin (EA2/067/11) and the Federal Medical Association of Baden Württemberg (B-F-2012-038). Data collection and storage procedures were approved by the Federal Commissioner for Data Protection and Freedom of Information.

## Results

We enrolled 109 case-patients out of 733 (15%) notified listeriosis patients (Fig 2) with a median age of 69 years (interquartile range, IQR 60–76); 60 (55%) were male. 103 (94%) case patients were hospitalized and four (4%) died. One-third (34%) of the case patients developed meningitis ( $n = 37$ ) and 20 (18%) developed sepsis. Sepsis was reported more frequently in cases older than 65 years compared to those who were younger (22% vs. 12%;  $\chi^2$   $p = 0.17$ ).

Cases included in the study were similar to notified cases not included with respect to sex, regional distribution of residence and proportion of sepsis. They were somewhat younger (median age: 69 years vs. 73 years; Wilcoxon rank-sum  $p = 0.02$ ) and a higher proportion developed meningitis than those not included in the study (32% vs. 21%;  $\chi^2$   $p = 0.01$ ). Confirmed listeriosis related death was 2.5-times more frequently reported for cases not included in the study (10%;  $\chi^2$   $p = 0.03$ ).

75 (72%) case patients reported at least one of the underlying diseases or immunosuppressive therapies associated with listeriosis (Table 1). Immunosuppressive therapies in the 3 months prior to illness onset were mentioned by 46 (44%) case patients, particularly in cases aged  $\leq 65$  years ( $n = 23$  [58%] vs.  $n = 23$  [36%] in cases  $> 65$  years;  $\chi^2$   $p = 0.03$ ).

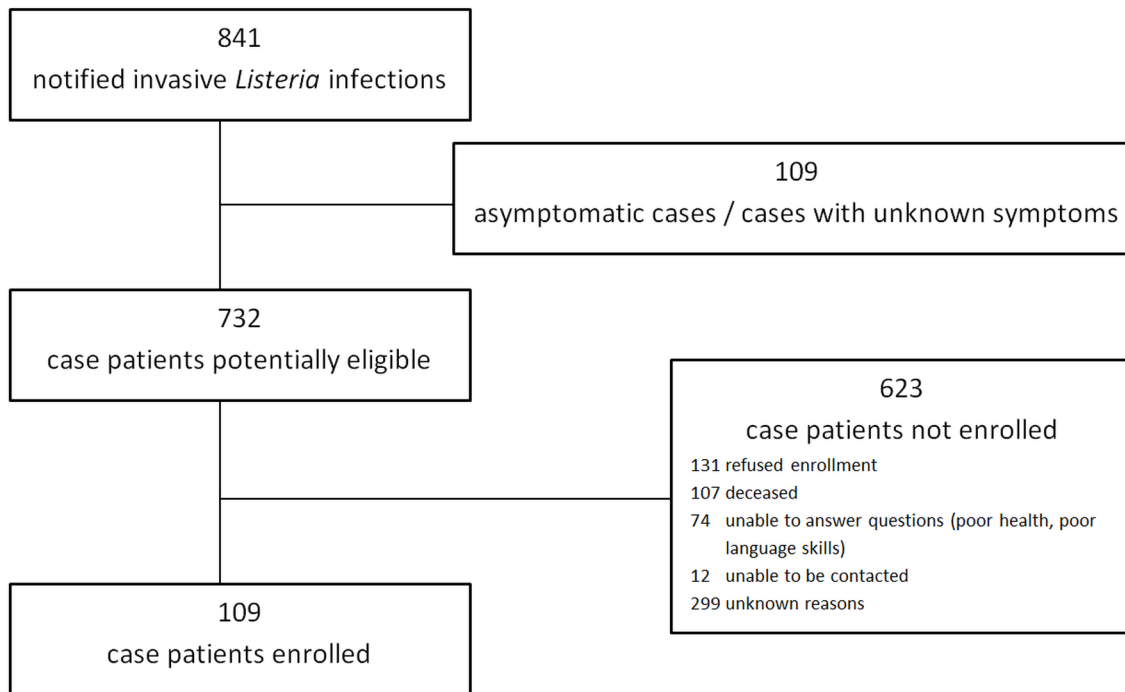


Fig 2. Enrollment of non-pregnancy associated listeriosis cases.

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During the study period, no listeriosis outbreak with evidence for a food vehicle was identified in Germany. The National Reference Center for *Salmonella* and other bacterial enteric pathogens did not find evidence of outbreaks by subtyping isolates voluntarily submitted for outbreak surveillance. Likewise, the statistical algorithms of the RKI did not flag a significant increase of listeriosis notifications during the time period.

Of 1990 recruited controls, eight had to be excluded due to their statement that they had suffered from listeriosis previously, resulting in 1982 control persons that were included in the study. Controls did not differ from cases with respect to age, sex and type of residence (Table 2), but had a higher proportion of high-level qualification than cases (33%; cases: 21%;  $\chi^2$   $p = 0.01$ ). A higher proportion of control persons knew about listeriosis (33%) than case patients prior to their illness (20%).

Knowledge about the disease was low in persons with immunocompromising diseases or who had received immunosuppressive therapy: only 13% of cases (7/54) and 10% of controls (58/589;  $\chi^2$   $p = 0.47$ ) knew transmission risks of listeriosis. Transmission via food was least known among persons under radiation therapy in the preceeding 3 months (1/21; 5%).

In univariable analysis, several underlying conditions were positively associated with listeriosis, including diabetes, renal disease or chemotherapy within the last 3 months as well as gastric acid suppression (Table 1). Furthermore, the following food-related exposures were significantly associated with increased odds of listeriosis ( $p < 0.05$ ): consumption of cold cooked sausages, pre-sliced cheese, packaged cheese, raw fermented spreadable sausages, cheese purchased in a supermarket or discounter, foods consumed in a hospital or nursing home, and foods consumed in a restaurant or canteen at least once a week. We found 23 exposures negatively associated with listeriosis (Table 3).

Based on the causal diagram (Fig 1), we identified age, sex, immunocompromising disease or immunosuppressive therapy, and gastric acid suppression as a minimally sufficient

**Table 2. Demographic characteristics of the study population in a case control study of risk factors for non-pregnancy associated listeriosis in Germany, 2012–2013.**

	Cases (n = 109)		Controls (n = 1982)		p-value*	
	n	%	n	%		
Age					0.295	
	≤65	42/109	38.5	666/1982	33.6	
	66–75	39/109	35.8	668/1982	33.7	
	≥76	28/109	25.7	648/1982	32.7	
Sex	Male	60/109	55.1	1101/1982	55.6	0.918
Educational background					0.021	
	Low	12/96	12.5	147/1918	7.7	
	Medium	64/96	66.7	1137/1918	59.3	
	High	20/96	20.8	634/1918	33.1	
Residence					0.620	
	Village	17/106	16.0	245/1824	13.4	
	Town	53/106	50.0	992/1824	54.4	
	Metropolis	36/106	34.0	587/1824	32.2	
Knowledge about listeriosis		20/101	19.8	655/1965	33.3	0.005
Immunocompromising diseases or therapies <sup>a</sup>					<0.001	
	No	29/104	27.9	1294/1890	68.5	
	Immunocompromising disease <sup>b</sup>	29/104	27.9	369/1890	19.5	
	Immunosuppressive therapy <sup>c</sup>	46/104	44.2	227/1890	12.0	

<sup>a</sup> mutual exclusive categories

<sup>b</sup> diabetes, renal disease, liver disease, autoimmune disorder, hematological cancer (within last 5 years), solid organ transplantation and/or splenectomy

<sup>c</sup> chemotherapy, radiation therapy, hemodialysis and/or immunosuppressive medication (all within the last 3 months)

\*  $\chi^2$  test

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adjustment set, which was forced into the multivariable model (Table 4). Immunosuppressive therapies and immunocompromising diseases remained strongly associated with listeriosis in the multivariable model. The odds of listeriosis in persons who had received immunosuppressive therapy in the last 3 months was approximately 9 times that of persons without any immunocompromising conditions (OR 8.75, 95% CI 4.91–15.58) and almost 3 times that of persons with immunocompromising diseases but without immunosuppressive therapy (OR 2.74, 95% CI 1.44–5.20). Furthermore, gastric acid suppression raised the odds for listeriosis by factor 3 (OR 2.96, 95% CI 1.40–6.25). The consumption of cold cooked sausages and preferred consumption of packaged cheese and sliced cheese were significantly associated with N-PA listeriosis.

The model specification was confirmed by the link test. We did not find significant interactions between the model variables.

The population attributable fraction (PAF) of the food-variables, i.e. consumption of non-reheated cold cooked sausages, preferably packaged cheese or preferably sliced cheese was approximately 25% each (Table 4). The total PAF for all identified risk foods was 58.9% (95% CI 49.8%–66.4%).

## Discussion

This population-based case-control study of risk factors for sporadic N-PA listeriosis in Germany yielded three main findings. First, reduced immunocompetency is the major risk factor



**Table 3. Food exposures statistically significantly associated with non-pregnancy associated listeriosis, Germany, 2012–2013: age-adjusted uni-variable logistic regression analysis.**

Consumption of ...*	Cases exposed		Controls exposed		OR	95% CI	p-value
	n	%	n	%			
Cold cooked sausages (e.g., frankfurters, wieners, bockwurst), not reheated	44/101	43.6	455/1976	23.0	2.54	1.69–3.81	<0.001
Pre-sliced cheese (in contrast to uncut cheese)	48/100	48.0	533/1948	28.4	2.34	1.56–3.51	<0.001
Packaged cheese (in contrast to unpackaged cheese)	59/100	59.0	803/1967	40.8	2.11	1.40–3.18	<0.001
Raw fermented spreadable sausages (e.g. Teewurst, Zwiebelmettwurst)	60/103	58.3	958/1975	48.5	1.51	1.01–2.26	0.043
Raw milk	2/106	1.9	254/1971	12.9	0.13	0.03–0.55	0.005
Ewe's cheese, goat cheese	16/101	15.8	956/1974	48.4	0.18	0.11–0.31	<0.001
Raw milk cheese	20/54	37.0	1034/1417	73.0	0.21	0.12–0.37	<0.001
Cold seafood (e.g., prawns, shrimps)	11/102	10.8	538/1980	27.2	0.31	0.17–0.59	<0.001
Mozzarella, feta cheese etc.	35/104	33.7	1101/1975	55.8	0.34	0.22–0.72	<0.001
Fresh cheese (e.g., cream cheese, cottage cheese, mascarpone)	43/102	42.2	1314/1962	67.0	0.35	0.23–0.52	<0.001
Carved and packaged raw fruit, fruit salads etc.	8/105	7.6	366/1978	18.5	0.37	0.18–0.78	0.008
Pasteurized milk	53/103	51.5	1451/1981	73.3	0.38	0.25–0.57	<0.001
Blue-veined cheese (e.g., roquefort, gorgonzola)	24/101	23.8	891/1976	45.1	0.38	0.24–0.61	<0.001
Acid curd cheese	23/99	23.2	808/1973	41.0	0.45	0.28–0.72	0.001
Red-smear cheese (e.g., Limburger, Romadur, Munster cheese)	23/100	23.0	745/1968	38.0	0.49	0.31–0.80	0.004
Semi-soft cheese (e.g., butter cheese, Esrom, Havarti)	33/98	33.7	978/1967	49.7	0.50	0.33–0.77	0.002
Cooked, roast or barbecued meat	86/103	83.5	1771/1980	89.4	0.55	0.32–0.95	0.034
White mould cheese (e.g., camembert, brie)	60/101	59.4	1420/1976	71.9	0.57	0.38–0.86	0.007
Dry-cured ham (e.g., Serrano ham, Parma ham)	70/104	67.3	1547/1979	78.2	0.57	0.37–0.86	0.008
Deli salads	41/103	39.8	1027/1977	52.0	0.59	0.40–0.89	0.012
Any cheese with negative association to listeriosis	92/103	89.3	1859/1966	94.6	0.48	0.25–0.94	0.031
Cheese from supermarket or discounter	100/105	95.2	1737/1971	88.1	2.66	1.07–6.60	0.035
Cheese from specialized shop	8/105	7.6	630/1959	32.2	0.17	0.08–0.36	<0.001
Meat/sausage from farmer's market, direct sale	6/104	5.8	481/1968	24.4	0.19	0.08–0.43	<0.001
Fish/seafood from travelling hawker, mail-order selling	2/103	1.9	163/1965	8.3	0.23	0.06–0.93	0.039
Fish/seafood from specialized shop	5/98	5.1	294/1654	17.8	0.25	0.10–0.62	0.003
Cheese from farmer's market, direct sale	6/104	5.8	369/1981	18.6	0.26	0.11–0.59	0.001
Fish/seafood from farmer's market, direct sale	9/101	8.9	374/1968	19.0	0.42	0.21–0.83	0.013
Foods supplied in a hospital, rest home, nursing home	11/108	10.2	78/1981	3.9	2.86	1.47–5.57	0.002
Foods purchased by other family or household members	85/108	78.7	1216/1980	61.4	2.27	1.41–3.63	<0.001
Foods supplied in a restaurant, canteen etc. at least once a week	32/104	30.8	423/1981	21.4	1.57	1.01–2.44	0.023
Foods from own purchase	86/109	78.9	1762/1982	88.9	0.43	0.26–0.70	0.002

\* within the last 4 weeks prior to onset of illness (cases) and prior to the interview (controls), respectively

Exposures not statistically significantly associated with N-PA listeriosis: Consumption of packaged meat and sausage products, salami, scalded deli meat, pâté, sliced cooked deli meat, sliced aspics, sliced blood sausage, raw minced meat, packaged fish/seafood, raw fish (sushi), smoked or graved fish, marinated fish, medium-hard/hard cheese, carved and packaged raw vegetables, meat/sausages from supermarket or discounter, butcher shop, wholefood shop, filling station/station kiosk, travelling hawker or mail-order selling, self-made meat/sausages, fish/seafood from supermarket or discounter, wholefood shop, self-made fish/seafood, cheese from wholefood shop, filling station/station kiosk, travelling hawker or mail-order selling, self-made cheese, and food supplied by delivery service (pizza service, meals on wheels etc.) or by assisted living.

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for listeriosis. Second, foods associated with listeriosis, i.e. cold cooked sausages and both packaged cheese and pre-sliced cheese, are ready-to-eat, usually have a long shelf-life, and were popular among the general adult population in Germany. Together, they accounted for 59% of the cases in Germany. Third, about 80% of cases had never heard of listeriosis prior to their illness

**Table 4. Risk factors for sporadic non-pregnancy associated listeriosis in Germany, 2012–2013: multivariable logistic regression analysis.**

	OR	95% CI	p-value	PAF (%)	95% CI (%)
Immunocompromising diseases or therapies <sup>a</sup>					
immunosuppressive therapy within the last 3 months	8.75	4.91–15.58	<0.001		
immunocompromising disease without immunosuppressive therapy	2.73	1.44–5.20	0.002		
none	Ref.				
Gastric acid suppression*	2.96	1.40–6.25	0.005	9.3	5.7–12.8
Consumption of (not-reheated) cold cooked sausages	2.60	1.56–4.35	<0.001	24.5	16.3–31.9
Preferred consumption of packaged cheese	2.09	1.25–3.49	0.005	30.7	14.7–43.8
Preferred consumption of pre-sliced cheese	2.19	1.31–3.66	0.003	27.2	14.4–38.0
Consumption of any cheese with negative association to listeriosis <sup>b</sup>	0.33	0.15–0.73	0.007		
Consumption of pasteurized milk	0.47	0.28–0.77	0.003		
Consumption of carved and packaged raw fruit, fruit salads etc.	0.27	0.11–0.72	0.008		
Consumption of deli salads	0.46	0.27–0.78	0.004		
Age*					
≤65	Ref.				
66–75	0.63	0.35–1.12	0.116		
≥76	0.54	0.29–1.01	0.056		
Female sex*	0.90	0.54–1.48	0.670		

<sup>a</sup> see Table 1

<sup>b</sup> raw milk cheese; ewe’s cheese, goat cheese; mozzarella, feta cheese; fresh cheese; blue-veined cheese; acid curd cheese; red-smear cheese; semi-soft cheese; white-mould cheese

\* were identified as the minimally sufficient adjustment set in a directed acyclic graph and thus included in the multivariable model

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and only 6% of the immunocompromised control population knew about the principally food-borne nature of the disease, indicating an as yet under-utilized potential for risk communication and disease prevention.

Our study confirmed [24–26] and quantified the outstanding role of reduced immunocompetency for development of N-PA listeriosis. 44% of cases had received an immunosuppressive therapy within the 3 months prior to illness onset and another 28% had an underlying immunocompromising disease, such as diabetes. The disease risk appeared to correlate with degree of reduced immunocompetency (assuming that patients who recently received immunosuppressive therapy were, on average, more immunocompromised than those with “only” immunocompromising disease(s)). Moreover, knowledge about listeriosis transmission pathways was low among immunocompromised persons and appeared to be particularly lacking among persons who had received chemotherapy or radiation therapy within the last 3 months. To our knowledge, dietary recommendations for prevention of food-borne infections do not exist for this subgroup of patients in Germany. Hence, healthcare institutions in Germany should be aware of the increased risk of immunocompromised persons for listeriosis if catering food for this population.

Gastric acid suppression was associated with listeriosis in former studies [7–9, 26] but failed to be an independent risk factor when controlled for underlying immunocompromising conditions [6, 27]. In our study, gastric acid suppression remained associated with listeriosis when adjusted for immunocompromising diseases and immunosuppressive therapy. Yet, all cases with gastric acid suppression and complete information for underlying conditions (n = 16) suffered also from at least one underlying immunocompromising disease (n = 7) or had received immunosuppressive therapy (n = 9). Thus, use of these drugs warrants particular care in already immunocompromised persons.

The identified ready-to-eat foods in this study, which are all frequently consumed in Germany, have not yet been considered risky foods in Germany, but they are plausible vehicles for sporadic N-PA listeriosis. Cold cooked sausages are heated during manufacturing but they are susceptible to post-production contamination until package [28]. They also support relatively rapid growth of *L. monocytogenes* under refrigerated storage conditions [29, 30] and *L. monocytogenes* is frequently detected in these products [31]. Furthermore, our finding of a significant association between listeriosis and consumption of cold cooked sausages that were not reheated before eating are consistent with a quantitative risk assessment of foodborne *L. monocytogenes* among ready-to-eat foods [32] in which non reheated frankfurters (a cold cooked sausage), were classified as a (very) high risk food.

Many different types of cheese—made of unpasteurized milk as well as from pasteurized milk—were identified as vehicle in listeriosis outbreaks [33] or have been the subject of an alert within the European Commission-operated Rapid Alert System for Food and Feed because of contamination with *L. monocytogenes* [34]. In keeping with this, we identified no single type of cheese but two retail forms of cheese, irrespective of the cheese type, that were statistically significantly associated with listeriosis. “Packaged cheese” combined various types of soft, semi-hard or hard cheese sold as whole pieces as well as pre-sliced. Notably, packaged cheese for self-service normally has an extended shelf-life and supports the growth of *L. monocytogenes* [35] after possible post-production contamination [36]. In contrast, cheese bought at counters is frequently cross-contaminated with mold and therefore has a shorter durability [37]. Hard cheese is the top-selling type of cheese in Germany and is often consumer-friendly offered as pre-sliced product, particularly in self-service retail, which accounts for 93% of cheese sales [38]. Slicing equipment is a known source for *Listeria* contamination in the food production and processing environment [39, 40]. Low level contamination of sliced cheese products with *L. monocytogenes* is not unusual [41] and sometimes even sufficient to cause outbreaks [42]. In our study, 18% of the control population reported daily consumption of sliced cheeses, a further 54% at least once per week. The popularity of pre-sliced and hard cheese in the general population may outweigh the apparently low prevalence of *L. monocytogenes* in hard cheese (0.4%), seldom in high concentrations (approx. 14% of positive samples), in a recent food survey in Germany [43].

The ability of *L. monocytogenes* to grow at low temperatures is well known [44]. Therefore, the European Commission Regulation on microbiological criteria for foodstuffs (EC No 2073/2005) requires that the concentration of *L. monocytogenes* in ready-to-eat foods, such as cold cooked sausages or pre-sliced and packaged cheeses, does not exceed 100 colony forming units per gram throughout the shelf-life. Our study results indicate (i) that this safety level may be insufficient to protect immunocompromised persons from acquiring listeriosis or (ii) that these ready-to-eat foods were consumed after the ‘use by’ date or the minimum durability date, thereby allowing *L. monocytogenes* to multiply to concentrations above the safety level, or both. That the retail form (e.g. pre-sliced/packaged) rather than a specific cheese type appeared to be important in this study, coupled with the observation that *L. monocytogenes* is infrequently present in these cheeses, lends credibility to the hypothesis that the time-interval between production and consumption of these cheeses, and possibly also of cold cooked sausages, plays a pivotal role for acquiring N-PA listeriosis in Germany. By eliminating the risk associated with any one of these foods, 25% of cases in Germany could be prevented (and 59% by eliminating the risk of all three) indicating their relevance for listeriosis in Germany and the large disease prevention potential.

Similar to other case-control studies of risk factors for sporadic listeriosis [6, 9], our study did not find a statistical association of listeriosis with many typical high risk foods, such as ready-to-eat fish products (graved/smoked fish) or certain types of unpasteurized cheese. On the

contrary, some typically risky foods (e.g. raw milk, red smear cheese, cold seafood) were negatively associated with listeriosis. We offer the following explanations for this finding. Firstly, many of the high-risk foods have become prominent through outbreaks (in other countries) and it is unclear whether outbreak-related risk factors equally apply to sporadic cases (in Germany). Secondly, some high-risk foods (e.g. gravled/smoked fish) are characterized by a high prevalence of *L. monocytogenes*. However, the overall prevalence of *L. monocytogenes* might not correlate well with the prevalence of pathogenic *L. monocytogenes*. Frequent occurrence of apathogenic or low virulent strains in retail foods and processing environment has been described [45, 46]. Furthermore, other factors such as food-processing before consumption, the food matrix's ability to support bacterial growth, and the time between production and consumption of the food may confound the association of a specific food-item with listeriosis.

Our study is subject to potential biases. We enrolled a smaller number of cases than planned which reduced the ability of the study to identify foods associated with listeriosis. Furthermore, cases in this study were somewhat younger and tended to have milder diseases than cases not included in the study and food consumption might depend on age. We aimed to include an age-adjusted representative sample of the German adult population as the reference population in this study, but our controls, as is common for telephone surveys [47], disproportionately contained persons with a high socioeconomic status (SES) (when approximated by educational background). Compared to the German microcensus of 2012, persons with highest educational background were 2-fold over-represented in our control population (33% vs. 16% at microcensus). As consumption of various food items, especially cheese types, was positively correlated with educational background in our large control population (data not shown), these foods were found to be rather negatively than positively correlated with N-PA listeriosis. Although we controlled for the confounding impact of SES analytically in the multivariable model, we cannot exclude that other factors, uncontrolled in this study, relate to consumption patterns through means other than SES.

We did neither enquire about specific types of cold cooked sausages nor about their package and shelf life. Thus, further studies are needed to better characterize the risk associated with this particular food item.

## Conclusions

Our study confirmed the outstanding role of reduced immunocompetency for acquiring N-PA listeriosis, and identified ready-to-eat foods as risk factors that are frequently consumed in Germany. These foods usually have an extended shelf-life, are biologically plausible, but have not yet been considered high-risk foods for listeriosis in Germany. To reduce their risk, immunocompromised persons, particularly those receiving immunosuppressive therapy, should consume packaged and pre-sliced cheese (and possibly cold cooked sausages) well before their 'use-by' date. It is possible that the microbiological criteria for *L. monocytogenes* in ready-to-eat foods are inadequate to protect persons with markedly reduced immunocompetency from acquiring listeriosis. Specific recommendations for this population should be formulated or amended and heed the findings of this study.

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## Author Contributions

Conceived and designed the experiments: AMB KS DW. Performed the experiments: KP. Analyzed the data: KP. Wrote the paper: KP DW. Designed and performed the population-based telephone survey: PS MW. Assisted with epidemiological interpretation: KS DW. Critically revised the manuscript: AMB PS MW KS.

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