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Serovar specific risk factors and clinical features of *Salmonella enterica* ssp. *enterica* serovar Enteritidis: a study in South-West Germany

M. J. KIST* AND S. FREITAG

Abteilung Medizinische Mikrobiologie und Hygiene, Institut für Med. Mikrobiologie und Hygiene, Klinikum der Universität Freiburg, Hermann-Herder-Str. 11, D-79104 Freiburg, Germany

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SUMMARY

Human salmonellosis in Germany has been an increasing problem since the 1980s, with a peak of 195000 reported cases in 1992. During the peak years, isolates of *Salmonella* Enteritidis (SE) predominated by far over other salmonella serovars (NSE) (80 *vs*. 20%). In a comparison of the clinical characteristics of 790 persons infected with SE to 175 persons infected with NSE, watery diarrhoea (OR 1·7) and high grade (> 39 °C) fever (OR 1·8) were independently associated with SE infection. When comparing possible risk factors for acquiring salmonella infection among patients with SE compared to those with NSE, consumption of raw eggs (OR 4·4; $P = 0{\cdot}0006$) was the most significant alimentary risk factor for SE infection, while travel outside Europe was negatively associated with SE infection (OR 0·08; $P = 0{\cdot}0001$). When comparing all patients with salmonella infection, regardless of serovar, with healthy controls, consumption of raw eggs (OR 30·3; $P = 0{\cdot}001$), of raw or undercooked eggs (OR 1·9; $P = 0{\cdot}003$), or having puppies, kittens or turtles (OR 6·8; $P = 0{\cdot}002$), were risk factors for salmonellosis.

INTRODUCTION

Since 1985, the annual incidence of human salmonellosis in Germany has increased with a dramatic acceleration since 1990. The incidence rose to over 100 reported cases per 100000 population, and peaked at 243/100000 in 1992 [1] when 195000 cases were reported. During this peak period, *S*. Enteritidis (SE) isolates accounted for about 80% of all human salmonella isolates [2]. This national epidemic was part of a pandemic increase [3]. After 1992 the incidence rates decreased continuously reaching 123/100000 in 1998 [4]. In Germany, as in other countries, more than 90% of human cases of salmonellosis appear as sporadic infections, although this often depends on the effectiveness of local investigation. Only a minority of infections are shown to have been derived from common source outbreaks [5]. On the other hand, most information on risk factors is derived from studies of outbreaks, when time and sources and routes of infection have been studied [6–8]. Most human infections with salmonella are from a food-borne source, but contact with infected persons, often in a family setting, contact with animals, often young animals, exposure during travel, as well as decreased host resistance, such as advanced age, underlying diseases or prior antimicrobial exposure are known risk-factors [9–11]. Investigations of the variables important in sporadic salmonellosis are based on reporting of individual cases, prevalence studies in potential animal and food reservoirs, detection of chains of infection, and case-control studies [12–15]. In the latter, identifying the most suitable control group is crucial [12]. Because the

* Author for correspondence.

peak incidence of salmonellosis in Germany was clearly correlated with an isolated increase of SE isolates, the present study concentrated on serovar-specific epidemiological risk factors and clinical characteristics. We compared epidemiological and clinical characteristics of the SE cases with those of cases infected with other non-SE salmonella (NSE) serovars. This design minimized selection and recall bias, while allowing a high probability of a comparable distribution of responders and non-responders. Furthermore it allowed identification of SE-specific risk factors in comparison to NSE cases. Potential risk factors for sporadic SE cases studied included age and socioeconomic status, foreign travel during 2 weeks before onset of symptoms, food history during 48 h before first symptoms, prior (1 month) ingestion of antimicrobials or antacids, preceding (2 weeks) infections in contact persons, and contact with farm animals and pets.

METHODS

Cases definitions: SE and NSE cases

Cases were defined as having SE-infection or NSE-infection if they met the following criteria: (1) lived in one of the three study areas, (2) presented with a diarrhoeic and/or febrile illness requiring medical care, (3) had a stool specimen sent during the study period (May 1991–January 1994) to the study centre laboratory or to microbiological laboratories located at the two affiliated study areas, and (4) had *S.* Enteritidis (SE) or a non-Enteritidis salmonella serotype (NSE) isolated, respectively. The study areas included the urban area of the city of Freiburg (200000 inhabitants) and surrounding smaller cities and rural villages (main study area), as well as two further areas (affiliated study areas). One further area was near Lake Constance and included a medium-sized city and rural villages, and the other area was near Stuttgart and included a medium-sized industrial city and smaller villages. Rural areas were defined as villages with less than 10000 inhabitants which included persons practising active farming. Urban areas were all study areas without active farming.

Cases resulting from obvious outbreaks were excluded. An outbreak was assumed if more than one case was identified from the same family or if clustering of cases was identified by the local health authorities. Salmonella carriers who were found during follow-up investigations in families were also excluded, as well as persons with typhoid or paratyphoid infections.

Case finding and data collection

Laboratory investigations

Among persons meeting the case definition 85% were identified by the study centre laboratory and 15% by laboratories in the affiliated areas. Seventy per cent of the investigated patients were outpatients and 30% inpatients, with a similar distribution for SE- and NSE-cases. Diarrhoea was reported by 97% of the SE and by 96% of the NSE-cases. Stool cultures and isolation of salmonellae, as well as biochemical identification and serotyping of isolates were done in the same laboratories. The main study laboratory and centre, the University Institute for Microbiology in Freiburg, serves the University Hospital and other hospitals of the region as well as practitioners and gastroenterologists from the main study area. As a public health laboratory, the same institute is responsible for follow-up and epidemiological field investigations in cases of transmissible enteric infections. Cases from the two affiliated study areas were identified by private laboratories.

Data collection

A standardized questionnaire was sent, together with the microbiological report, to the physician responsible for the individual patient. After obtaining informed consent and interviewing the patient, the questionnaire was completed by the physician and sent to the study centre. If necessary, missing data were collected from the physician, or with permission of the physician, from the patient or, in case of children, from their parents. As a rule, patients were interviewed during the first week after the first stool investigation. The questionnaire asked for demographic and socioeconomic characteristics including place of residence, foreign travel during 2 weeks before onset of symptoms, preceding (2 weeks) fever or diarrhoea in contact persons, prior (1 month) consumption of antacids or antimicrobials, consumption of various food during 48 h before onset of symptoms, and contact with domestic and farm animals and pets. The physicians were asked to answer questions concerning underlying diseases and clinical features of the enteric illness. Clinical features included onset of symptoms, duration of symptoms, signs and findings suggesting septicaemia, urinary tract involvement, meningitis, osteomyelitis, orchitis, pneumonia, fever and duration of fever, vomiting, presence and duration of diarrhoea, stool characteristics, vomiting, pains, and antimicrobial therapy.

Comparison groups

Two groups were used for comparison with SE cases: (1) NSE cases with salmonellosis not caused by *S*. Enteritidis (see above), and (2) a systematically selected population-based group. This latter group was recruited from August 1993 to February 1994 from the Freiburg study area only, using the telephone directories for Freiburg and its surrounding areas. As a rule the first phone number of each row in the phonebook was called. All phone calls were completed between 18.00 and 20.00 h. The person who answered the phone was interviewed. The persons telephoned were asked for age and if they consented to answer the questions. About 30% of the persons called refused to participate. All consenting persons were interviewed, by telephone, by the same interviewer, using a standardized questionnaire, identical to the case questionnaire, with appropriate modifications concerning the clinical picture. For children less than 15 years old, one of the parents was questioned. Individuals reporting symptoms compatible with enteric infection were excluded from the analysis. To avoid unnecessary disproportion between cases and controls, frequency matching by age group and sex was done. Controls in a given age and sex category were interviewed until the desired quota in that age and sex category was reached and then no further controls in that category were eligible to be interviewed. For residence in urban or rural areas, respectively, matching was done by using telephone directories of the appropriate areas. To establish the healthy population-based comparison group, a total of 256 individuals were recruited.

Denominators

Age specific rates were calculated for the whole study period (29 months) using population data as of 1 January 1992 for the Freiburg study area, provided by the Statistisches Landesamt Baden-Württemberg.

Statistical methods

Data were collected in d-Base III, and data analysis done using the SAS program (SAS Institute Inc., Cary, NC, U.S.A.). Analysis was performed to compare exposure between case and comparison groups with stratified data using univariate analysis (PROCFREQ), as well as applying Logistic Regression Analysis (PROCLOGISTIC). The test procedures were Pearson–χ^2 for contingency tables and WALD–χ^2 for logistic regression. The same methods were used to compare clinical features between the *S*. Enteritidis and the non-*S*. Enteritidis groups.

RESULTS

From August 1991 to January 1994, 1704 salmonella cases with stool specimens submitted to the central study laboratory were identified; 1357 (80%) of whom had *S*. Enteritidis (SE-group) and 347 (20%) of whom had salmonella of non-*S*. Enteritidis serovars (NSE-group). The age-specific isolation rates over the whole study period are shown in Table 1. Isolation rates of both, the SE and the NSE salmonella cases peaked in the 0–4 year old age group. Questionnaires were completed and returned for a total of 688 SE-patients (51%) and for a total of 158 NSE-patients (46%). The difference was not statistically significant. From the external study centres, a total of 119 questionnaires (SE:102; NSE 17) were returned from cases identified. In total, data from 790 SE-cases were compared with 175 NSE-controls. In five questionnaires, the age of the patient was missing and the respective data were excluded from the age-related analysis. Cases with completed questionnaires were equally distributed between rural and urban areas. Seventy per cent of the cases were outpatients and 30% were inpatients at the time of specimen collection. The SE-group and the comparison NSE-group did not differ significantly by age, urban or rural residence, or by in- or outpatient status.

Clinical features

Clinical features of the SE- and the NSE-group were similar in relation to; duration of illness more than 7 days (46 *vs*. 42%); the presence of fever $\geqslant$ 38 °C (81 *vs*. 79%); abdominal pain (73 *vs*. 73%); diarrhoea (97 *vs*. 96%); diarrhoea lasting longer than 7 days (32 *vs*. 29%); mucoid stools (43 *vs*. 44%); extraintestinal symptoms including arthralgia (26 *vs*. 22%). Both groups were equally likely to have been treated with antimicrobials (16 *vs*. 17%). By univariate analysis, watery diarrhoea, without blood and mucous, was significantly more frequent among persons with SE-

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Table 1. *Isolation rates of salmonellae (SE and NSE) in different age groups. Study period: 29 months*

	0–4 years	5–14 years	15–59 years	60+ years	Total
Denominator (1/1/1992)	23698	41275	274848	80038	419859
SE (*n*)	337	265	608	147	1357
SE (isolation rate)	1422/100000	642/100000	221/100000	184/100000	327/100000
NSE (*n*)	110	51	144	42	347
NSE (isolation rate)	464/100000	124/100000	52/100000	52/100000	83/100000
All salmonella cases (*n*)	447	316	752	189	1704
All salmonella cases (IR)	1866/100000	766/100000	273/100000	236/100000	410/100000

Table 2. *Case-control-study of patients with* S. *Enteritidis (SE)-infection versus patients with non-*S. *Enteritidis (NSE)-infection: multivariate logistic regression analysis (LR)* of risk factors for clinical signs and symptoms*

Clinical signs and symptoms	Risk factor	OR	*P*-value
Watery diarrhoea	SE-infection†	1·7	0·0027
	Age group 40+ yr	3·6	0·0008
Fever > 39 °C	SE-infection	1·8	0·022
	Consumption of raw eggs§	2·2	0·0002
	Age group 0–4 yr	5·2	0·0001
	Age group 5–14 yr	3·1	0·025

* The following variables were included in the LR: gender; age groups (0–4, 5–14, 15–39, 40+ years); salmonella group (SE or non-SE-serotypes); consumption of any eggs; consumption of raw eggs; consumption of raw or undercooked eggs; consumption of raw red meat; consumption of raw or undercooked red meat. Age groups 15–39 were used as reference. Since backward elimination was applied, reference groups for each other factor included were those of all other risk levels.
† Cases with *S.* Enteritidis-infection.
§ During 48 h before onset of symptoms.

infection than among those with NSE (87 *vs.* 79%; OR 1·8 [1·1–2·8], $P = 0·01$). The logistic regression model included the following parameters: gender, age-group (0–4, 5–14, 15–39, 40+ years), salmonella-serovar (SE *vs.* NSE), and various food items ingested during 48 h before onset of symptoms (Table 2). Watery diarrhoea was independently associated with SE-infection (OR 1·7), but also with age greater than 39 years, independent of the salmonella-serovar (OR 3·6). High-grade fever over 39 °C was significantly associated with SE-infection (58 *vs.* 51%, OR 1·75) younger age, particularly age $\leqslant$ 4 years (OR 5·2), and previous consumption of raw eggs (OR 2·15) (Table 2).

Epidemiological risk factors

Univariate analysis, comparing the travel histories of both groups, showed that patients with *S.* Enteritidis-infection significantly less frequently reported travelling during the 2 weeks before onset of symptoms (OR 0·3), particularly outside of Europe (OR 0·1). Frequency of travelling within Europe was not significantly different between both salmonella groups. Of those with a recent travel history, 20% of the SE-group and 57% of the NSE-group reported recent overseas travel (OR 0·2) (Tables 3, 4). The socio-economic and demographic characteristics of both groups were not significantly different: 50% of the SE-group and 47% of the NSE-group resided in rural areas, and 53 or 49% respectively reported secondary and higher education (data not shown). Both groups showed only slight differences in total egg consumption of any kind during 48 h before onset of symptoms (SE 61% *vs.* NSE 51%) (Table 3). Among those with recent egg consumption, intake of raw or undercooked eggs were significantly more frequently reported by the SE-group (76 *vs.* 40%;

Table 3. *Case-control-study of patients with* S. *Enteritidis* (*SE*)-*infection versus patients with non*-S. *Enteritidis* (*NSE*)-*infection: single variable analysis of epidemiological risk factors*

	Proportion exposed			
Risk factors	SE*	NSE*	OR (CI)	*P*-value
Travelled†	55/778 (7·1%)	37/171 (21·6%)	0·28 (0·18–0·43)	0·0001
Travelled within Europe	44/778 (5·7%)	16/171 (9·4%)	0·6 (0·3–1·1)	n.s.
Travelled outside Europe	11/778 (1·4%)	21/171 (12·3%)	0·1 (0·05–0·22)	0·001
Consumed any eggs‡	391/640 (61%)	67/132 (51%)	1·5 (1·1–2·2)	0·03
Consumed raw eggs	128/352§ (36%)	13/65§ (20%)	2·3 (1·2–4·4)	0·016
Consumed raw or undercooked eggs	267/352 (75%)	26/65 (55%)	4·7 (2·7–8·2)	0·0001
Consumed poultry meat	120/622 (19%)	30/120 (25%)	0·7	n.s.
Consumed red meat	437/636 (69%)	79/130 (61%)	1·4	n.s.
Consumed raw milk	67/659 (10%)	16/138 (12%)	0·9	n.s.

* Case-control-study comprised 790 SE-cases and 175 NSE-cases but not all respondents answered each question.
† During 2 weeks before onset of symptoms.
‡ All food items consumed during 48 h before onset of symptoms.
§ Denominator subgroup consisting of patients who reported consumption of any eggs and provided further details.

Table 4. *Case-control-study of patients with* S. *Enteritidis* (*SE*)-*infection versus patients with Non*-S. *Enteritidis* (*NSE*)-*infection: odds ratios of epidemiological risk factors for SE infection compared to NSE infection, when adjusted for variables* in the multivariate logistic regression analysis* (*LR*)§

Risk factors (serotype-specific)	OR	*P*-value
Not travelled†	2·2	0·01
Travelled outside Europe†	0·08	0·0001
Consumption of raw eggs‡	4·4	0·0006
Consumption of raw or undercooked eggs	2·2	0·001
Consumption of any eggs	1·0	1·0
Consumption of poultry meat	1·2	0·4 (n.s.)
Consumption of raw or undercooked red meat	0·6	0·3 (n.s.)

* The following variables were included in the LR: (a) for calculating travel associated risk: no travel; travel to European countries; travel overseas; gender; age groups (0–4, 5–14, 15–39, 40+ years); (b) for calculating food associated risk: consumption of raw eggs, consumption of raw or undercooked eggs, consumption of any eggs, consumption of poultry meat, consumption of raw red meat, consumption of raw or undercooked red meat, age groups (0–4, 5–14, 15–39, 40+ years). Since backward elimination was applied, reference groups for each risk factor included were those of all other risk levels.
† During 2 weeks before onset of symptoms.
‡ Each consumption during 48 h before onset of symptoms.
§ Table is a summary of two (a, b) LR models; n.s., statistically not significant.

OR 4·7) (Table 3). This association was also true for consumption of raw eggs (OR 2·3). No significant differences between both groups were observed concerning consumption of poultry (19 *vs.* 25%), red meat (69 *vs.* 61%) and raw milk (10 *vs.* 12%) (Table 3). Previous diarrhoea in contacts were present in 34 and 32% of index patients of SE- and NSE-patients respectively (Table 5). There were no significant differences in frequencies of holding farm animals or pets, nor the reported frequencies of taking antacids (2 *vs.* 3%) or antimicrobials (7 *vs.* 9%) (Table 5). Frequencies of preceding chronic enteritis, gastrectomy or immunosuppression were also nearly equally distributed (data not shown).

Table 5. *Case-control-study of patients with* S. *Enteritidis* (*SE*)-*infection or patients with non*-S. *Enteritidis* (*NSE*)-*infection versus healthy controls** : *single variable analysis*

	S. Enteritidis†				Non-*S.* Enteritidis†			
Exposure	Cases	Controls	OR (CI)	*P*-value	Cases	Controls	OR (CI)	*P*-value
Any egg	391/640 (61%)	99/256 (39%)	2·5 (1·8–3·4)	0·0001	67/132 (51%)	99/256 (39%)	1·6 (1·1–2·5)	0·023
Raw or under-cooked eggs	267/352‡ (75%)	36/99‡ (36%)	5·5 (3·4–8·9)	0·001	26/65‡ (40%)	36/99‡ (36%)	1·2 (0·6–2·2)	n.s.
Raw eggs	128/352‡ (36%)	1/99‡ (1%)	56 (7·7–406)	0·001	13/65‡ (20%)	1/99‡ (1%)	24·5 (3·1–193)	0·001
Any poultry	120/622 (19%)	52/252 (21%)	0·9	n.s.	30/120 (25%)	52/252 (21%)	1·3	n.s.
Red meat	437/636 (69%)	179/255 (70%)	0·9	n.s.	79/130 (61%)	179/255 (70%)	0·7	n.s.
Raw milk	67/659 (10%)	24/256 (9%)	1·1	n.s.	16/138 (12%)	24/256 (9%)	1·1	n.s.
Previous diarrhoea in household contacts§	236/696 (34%)	35/253 (14%)	3·2 (2·2–4·7)	0·0001	46/142 (32%)	35/253 (14%)	3·0 (1·8–4·9)	0·0001
Chickens or ducks	51/721 (7%)	8/253 (3%)	2·3 (1·1–5·0)	0·03	17/155 (11%)	8/253 (3%)	3·8 (1·6–9·0)	0·001
Puppies < 6 months	27/557 (5%)	1/256 (0·5%)	13·0 (1·8–96)	0·001	3/129 (2%)	1/256 (0·5%)	6·1 (0·6–59)	n.s.
Kittens < 6 months	27/560 (5%)	3/256 (1%)	4·3 (1·3–14)	0·01	6/125 (5%)	3/256 (1%)	4·3 (1·0–17)	0·03
Antimicrobials	57/772 (7%)	20/256 (8%)	0·9	n.s.	16/172 (9%)	20/256 (8%)	1·2	n.s.
Antacids	17/763 (2%)	4/256 (2%)	1·0	n.s.	5/164 (3%)	4/256 (2%)	2·0	n.s.

* Matched for age groups (0–4, 5–14, 15–39, 40+ years), gender and urban or rural area of residence.
† Case-control-study comprised 790 SE-cases and 175 NSE-cases but not all respondents answered each question.
‡ Denominator subgroup consisting of patients who reported consumption of any eggs and provided further details.
§ During 14 days before onset of symptoms in the case.
Turtles were omitted from the table because of small numbers in the SE and NSE group, and absence from the control group.

Table 6. *Case-control-study of patients with salmonellosis* (S. *Enteritidis or Non*-S. *Enteritidis*) *versus healthy controls**: *odds ratios of epidemiological risk factors for salmonella infection, when adjusted for variables*† *in the multivariate logistic regression analysis* (*LR*)

Risk factors	OR	*P*-value
Having puppies, kittens or turtles	6·8	0·002
Consumption of raw eggs‡	30·3	0·001
Consumption of raw or undercooked eggs	1·9	0·003
Consumption of any eggs	1·2	0·4 (n.s.)
Consumption of poultry	1·1	0·6 (n.s.)
Consumption of raw or undercooked meat	1·7	0·4 (n.s.)

* Group-matched for age groups (0–4, 5–14, 15–39, 40+ years), gender and urban or rural area of residence.
† The following variables were proved in the LR: consumption of any eggs, consumption of raw eggs, consumption of raw or undercooked eggs, consumption of any red meat, consumption of raw or undercooked red meat, consumption of poultry meat, holding of chickens or ducks, holding of puppies, kittens or turtles.
‡ Each consumption during 48 h before onset of symptoms.

Travelling as a risk factor was further analysed applying a logistic regression model, including the parameters 'no travel', 'travel within Europe', 'travel outside Europe', age and gender. The analysis showed 'no travel' significantly associated with SE-infection (OR 2·2; $P = 0{\cdot}01$), whereas 'travel outside Europe' was clearly negatively correlated (OR 0·08; $P = 0{\cdot}0001$) (Table 4). Further analysis of alimentary risk factors was done using a logistic regression model which included: age group, consumption of any eggs, raw eggs, raw or undercooked eggs, consumption of raw or undercooked red meat, and consumption of poultry meat. The consumption of raw or undercooked eggs (OR 2·2; $P = 0{\cdot}001$) and even more the consumption of raw eggs (OR 4·4; $P = 0{\cdot}0006$) were associated with the SE-infection, whereas no differences were found in the frequencies of eating eggs of any kind, of poultry meat or of raw and undercooked meat (Table 4).

Patients with salmonellosis (SE and NSE) *vs*. healthy controls

Univariate analysis showed that egg consumption, regardless of the mode of preparation, was significantly more frequently reported by the SE-group (61%) than by healthy controls (39%) (OR 2·5; $P < 0{\cdot}0001$). The difference was less pronounced for the NSE-group (51%) (OR 1·6; $P = 0{\cdot}023$). However, raw eggs were much more likely to have been reported by both persons with SE and non-SE compared to healthy controls (OR 56 and 24·5 respectively) (Table 5). There was no difference between the groups in consumption of poultry, red meat or raw milk (Table 5). Preceding diarrhoea in contact persons of index cases was reported in 34% of the SE- and in 32% of the NSE-groups. This was significantly more frequent than in healthy controls (14%) (Table 5). Whereas the univariate analysis revealed for both groups with salmonella infection a statistically significant association with having kittens at home, holding of puppies or kittens was only a rare event in all groups. Consumption of antimicrobials (7, 9 and 8%) and/or antacids (2, 3 and 2%) during 4 weeks before onset of symptoms or completing the questionnaire was rarely reported in all groups and was not a risk factor (Table 5). Travelling was not compared between cases with salmonella infections and healthy controls because controls had been interviewed predominantly during winter months and, thus, travel histories were not comparable to cases who had been interviewed throughout the year.

Multivariate analysis was done using a logistic regression model which included: consumption of any eggs, raw eggs, raw and undercooked eggs, consumption of any as well as of undercooked red- and poultry meat, having ducks or poultry as farm animals or having turtles, puppies or kittens as pets. In this model only having puppies, kittens or turtles (OR 6·8), consumption of raw eggs (OR 30·3), and consumption of raw or soft-boiled eggs (OR 1·9) were significant epidemiological risk factors for salmonellosis regardless of the serovar (Table 6). Concerning consumption of raw and undercooked eggs, univariate analysis of SE and NSE cases in comparison to healthy controls had already shown that there existed significantly higher risks for SE cases (Table 5). Because SE cases accounted for 80% of all salmonella cases, the results of the logistic regression analysis concerning egg borne risks were mostly influenced by these SE cases.

DISCUSSION

The main goal of the study was to identify serovar-specific risk factors which might explain the large increase of human salmonellosis in Germany between 1988 and 1992, which was manifested by an increase of sporadic cases of *S.* Enteritidis infections. Therefore, the central case-control study was conducted using persons with clinically apparent salmonellosis due to the serovar *S.* Enteritidis (SE) compared to persons with salmonellosis due to other serovars (NSE). This design of comparing groups with a clinically indistinguishable gastrointestinal infectious disease of bacterial origin, differing only in respect of the causative agent, has the advantage that it can reduce the possible impact of recall, selection or information bias [16]. Whereas in most case-control studies ill patients are compared with healthy controls, a design comparable to the present study was also used by Hedberg and colleagues, 1993 [14] and by Schmidt and colleagues, 1996 [15]. In the present study, as a second comparison, data from all patients with salmonellosis regardless of serovar were, in addition, compared to a healthy control group. Despite the fact that this healthy control group was selected from the main study population and was matched by age group, gender and rural or urban residence, several factors could have affected the validity of the results. First, potential selection bias due to non-response among the controls was difficult to evaluate. Healthy controls could have also had less sensitive recall or a different previous information level concerning possible risk factors of salmonellosis, thus introducing possible information bias. Finally, a possible interviewer bias has to be taken into account. Taken together, the comparison of SE and NSE case groups may be less biased than comparison of persons with salmonellosis and healthy controls.

The main findings from the SE-patients *vs.* NSE-patients case-control study were: (1) that sporadic *S.* Enteritidis infections in South-West Germany, as a rule, are not acquired from abroad; (2) that the consumption of raw or undercooked eggs are the most important risk factor for sporadic *S.* Enteritidis infections compared to other enteric salmonella infections; (3) independent of the patients' age, SE infection was more likely to have been characterized by predominantly watery diarrhoea and fever over 39 °C. All these findings were confirmed as independent risk factors by multiple logistic regression analysis. If, on the other hand, all cases of salmonellosis were taken together and compared with a healthy comparison group, prior diarrhoea in household contacts as well as having puppies, kittens or turtles appeared to be associated with salmonella infection, regardless of serotype. Despite the fact that human salmonellosis in Germany is notifiable as a communicable disease, there are no official data available concerning the ratio of imported infections. Only 7% of SE-patients but 22% of NSE-patients recalled any travelling during the 2 weeks before onset of symptoms and only 1·4% or 12% respectively reported overseas travel, (i.e. areas outside of Europe). This is much less than in a Swiss study, where 20·4% of the SE-group and 55·5% of the NSE-group reported travelling during 3 days prior to illness [15]. Such differences in results possibly are explained by difference in age groups. Whereas only persons over the age of 14 years were enrolled in the Swiss study, the study presented here included all age groups, particularly infants, who have a lower probability of recent travel. However, because the German patients were asked to recall travel during 2 weeks and not merely 3 days before onset of symptoms, the impact of travelling as a risk factor might, compared to the Swiss study [15], be rather overestimated. Nevertheless, both studies showed that SE infections are less frequently associated with travel than those due to other serotypes. It appears a minority of human salmonellosis cases in Germany are acquired outside the country, and that, among the salmonellosis cases, SE-infections are strongly negatively associated with recent travel history.

Concerning the vehicle of infection, the present study clearly shows that consumption of raw or undercooked eggs independently increases the risk of symptomatic SE-infection. This is obviously a specific association because after controlling for various confounders in the logistic regression analysis, in contrast to consumption of raw or undercooked eggs, consumption of any eggs was not associated with the disease. Raw eggs and egg-products have already been shown as the most important risk factors in other comparable case-control studies, like that of Hedberg and colleagues [14], where an association of *S*. Enteritidis but not *S*. Typhimurium was shown with food containing eggs with a fluid or soft egg yolk. Eating food containing raw or undercooked eggs was also identified as a SE-specific risk factor in the Swiss study [15], where, comparable to our findings, an increased risk was associated with eating raw egg-, rather than soft-boiled-egg-containing food. Cowden and colleagues [13], by comparing SE-cases with healthy controls, also found raw-egg containing food significantly associated with cases. Eggs are an excellent vehicle for the spread of *S*. Enteritidis: *S*. Enteritidis in eggs remain viable for weeks both in the egg-white and especially in the yolk where they are able to multiply rapidly [17, 18]. Eggs are very frequently consumed in Germany (about 200–300 per person per year) and, in contrast to poultry, which usually is thoroughly cooked, according to traditional practice, eggs are often consumed raw or only lightly heated, i.e. in soups, sauces or cakes. Since 1993, when refrigeration of eggs after 18 days was made mandatory by law [19], the incidence of human salmonellosis in Germany decreased continuously together with the consumption of eggs [20, 21]. The association of this intervention measure with decreasing numbers of reported infections further suggests the importance of eggs as an essential risk factor for *S*. Enteritidis salmonellosis. In contrast to the impact of raw or undercooked eggs as a *S*. Enteritidis associated risk factor, consumption of poultry meat was not significantly associated with infection. This is in agreement with the findings of Coyle and colleagues [22] in Wales and with those of Hedberg and colleagues [14] in the United States. It might be explained, in part, by appropriate food handling, because the role of poultry meat as source of salmonella infections is better known in the population. A further reason might be that *S*. Enteritidis predominantly infects visceral organs of poultry, which are not consumed or only consumed after thorough cooking. Neither red meat nor raw-milk drinking appeared to be significant risk factors for *S*. Enteritidis infection. The latter may be explained by the very low frequency of salmonella contamination in raw milk in South-West Germany, where, in only 1 of 550 samples, *S*. Typhimurium was found [23]. A negative association between the *S*. Enteritidis-infection and consumption of red meat, especially hamburgers, was also observed in the Swiss study [15] as well as in the English study by Cowden and colleagues [13]. In contrast to these findings, the American study of Hedberg and colleagues [14] identified undercooked hamburgers as an *S*. Enteritidis-associated risk factor.

The frequency of preceding infections in household contacts and of having animals were not different in the SE and the NSE-groups. From these results it may be concluded that the risk of acquiring salmonellosis by human-to-human spread is similar for both SE and NSE salmonellas.

Both groups of patients, SE- and NSE-infected, in general, exhibited similar clinical courses, with the exception that watery diarrhoea without blood and mucous as well as fever of more than 39 °C was significantly more often reported from SE-infected individuals. This finding was independent of age. High fever might be due to an effect of generally higher infective doses of raw egg-borne *S*. Enteritidis infections. This assumption is supported by the fact that high fever was also found independently associated with consumption of raw eggs. An association between the probable infective dose and clinical course in patients involved in a *S*. Enteritidis outbreak was also observed by Mintz and colleagues [24].

In conclusion, the present study has identified raw or undercooked eggs both as the main risk factor for infection and as a serotype-specific source of sporadic *S*. Enteritidis-infections. These SE infections are more frequent than other salmonelloses, are infrequently acquired abroad, and seem, as compared to NSE-associated enteric infection, more often associated with high fever and a predominantly watery diarrhoea. Preventive measures in producing, storing and handling eggs as well as increased knowledge in the population may have resulted in a decrease in human salmonellosis in Germany, as well as in Switzerland [15], but 'despite an encouraging decrease in reported salmonella infections since 1992, continued efforts in prevention are still necessary' [15].

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