Epidemiol. Infect. (1998), **121**, 561–567. Printed in the United Kingdom

Risk factors for the occurrence of sporadic *Salmonella enterica* serotype *enteritidis* infections in children in France: a national case-control study

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(*Accepted 23 July 1998*)

SUMMARY

To determine risk factors associated with the occurrence of sporadic cases of *Salmonella enteritidis* infections among children in France, we conducted a matched case-control study. Cases were identified between 1 March and 30 September 1995. One hundred and five pairs of cases and controls matched for age and place of residence were interviewed. In the 1–5 years age group, illness was associated with the consumption of raw eggs or undercooked egg-containing foods (OR 2·4, 95% CI 1·2–4·8). Storing eggs more than 2 weeks after purchase was associated with *Salmonella enteritidis* infection (OR 3·8, 95% CI 1·4–10·2), particularly during the summer period (OR 6·0, 95% CI 1·3–26·8). Cases were more likely to report a case of diarrhoea in the household 10–3 days before the onset of symptoms, particularly in the age group $\leqslant 1$ year ($P = 0{\cdot}01$). This study confirms the link between eggs and the occurrence of sporadic cases of *Salmonella enteritidis* among children, highlights the potential role of prolonged egg storage and underlines the role of person-to-person transmission in infants.

INTRODUCTION

Between 1986 and 1995 cases of *Salmonella enterica* serotype *enteritidis* (*Salmonella enteritidis*) infections increased dramatically in France [1]. Foodborne outbreaks of *Salmonella enteritidis* were found to be associated with the consumption of raw or undercooked eggs or egg products, particularly those bought in farms [2]. However, no studies have been conducted to identify more precisely food vehicles associated with sporadic cases of *Salmonella enteritidis*. Young children (< 5 years of age) who suffer more often a severe illness, bacteremia and dehydration, have an incidence of salmonella infection greater than adults [1, 3].

Despite the preventive measures implemented at the level of production and distribution of eggs and egg products in 1989, the number of *Salmonella enteritidis* isolates recorded in France has continued to rise [1, 4]. Other approaches such as education programmes to parents (e.g. storage, cooking of eggs) may, therefore, be needed as complementary measures to limit the transmission of the infection to young children. However, there are few epidemiological data on the influence of storage, preparation and consumption of eggs, egg products and poultry meat on the risk of sporadic *Salmonella enteritidis* infection among children. In addition, person-to-person transmission of *Salmonella enteritidis* has not been well documented. In an attempt to answer these questions, we conducted a national case control study among children $\leqslant 5$ years of age in France in 1995.

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SUBJECTS AND METHODS

We used a matched case-control study design. A sporadic case was defined as a child $\leqslant 5$ years, who resided in France (overseas districts and territories were excluded), and had presented with fever (temperature > 38 °C) or diarrhoea ($\geqslant 3$ loose stools per day for more than 1 day) associated with the isolation of *Salmonella enteritidis* from stool or blood, between 1 March and 30 September 1995.

Cases were identified each week by reviewing isolates received by the National Reference Centre for *Salmonella* and *Shigella* (NRC). The NRC receives salmonella isolates for serotyping from about one third of the 4000 French microbiology laboratories [1]. The laboratories that send salmonella isolates to the NRC are equally distributed through the districts of France (data not shown).

Since the incidence of *Salmonella enteritidis* infections and the probability of exposure to the vehicle are both thought to be linked to the place of residence as well as to age, controls were matched to cases according to age (2 age groups; $\leqslant 1$ and > 1–5 years) and place of residence. For each case, one control of the same age group and the same city or county, of residence who had had no gastro-intestinal symptoms during the month prior to onset of illness in the case was searched for. Controls were found among the patients of the case physician. The case physician was asked to select the first patient to meet the above-mentioned criteria. When controls could not be obtained from the physician, they were sought from the Minitel telephone directory which is an equivalent to random digit dialling by area of residence.

The investigator interviewed parents of cases and controls by telephone, using a pre-tested standardized questionnaire. Besides information on symptoms and demographic characteristics, the questionnaire investigated the following exposures during the month prior to the onset of illness in the case: consumption, purchase, storage and preparation habits of eggs and egg products, poultry meat and beef meat, contact with persons who had diarrhoea, day-care or nursery attendance. Eggs consumption was further characterized according to place of purchase (farm, local market, supermarket), place and length of storage (refrigerator, number of weeks), cooking procedures (raw, lightly cooked, fried, soft or hard boiled, scrambled), and frequency of consumption. Parents were interviewed about their own consumption and cooking habits. Information was also collected on underlying immunosuppressive conditions, prematurity, breast feeding, use of medications such as antibiotics in the month prior to disease and the presence of a pet at home.

In order to detect an odds ratio of 2·5 with a 25% exposure among controls ($\alpha = 5\%$, $\beta = 80\%$), we needed 94 pairs of cases and controls. The study was stratified by period of *Salmonella enteritidis* infection occurrence: 'summer period' (June to September) and 'non-summer period' (March to May) with a greater sampling fraction of cases included from March to May than between June to September.

Analysis was done with Epi-Info 6 for single variable analysis. Categorical data were analysed by calculating univariate matched odds ratios (OR) and their 95% confidence intervals (95% CI). Statistical significance was assessed by McNemar's χ^2 test for dichotomous variables and the matched t test for continuous variables. Exposures found to be associated with illness by univariate analysis (P value $< 0{\cdot}2$) were included in a conditional logistic-regression model through stepwise deletion of variables (EGRET, SERC Inc., Seattle, WA, USA). Since the season of inclusion was identical for each case-control pair, we built three models, one without including the season, one restricted to the summer period and one to the non-summer period.

RESULTS

Of the 260 cases (isolation of *Salmonella enteritidis*, age $\leqslant 5$) recorded by the NRC during the study period, 198 were chosen at random, of which 33 were not eligible for the study (delay between diagnosis and serotyping longer than 1 month, $n = 3$; asymptomatic, $n = 6$; residence or travel outside the French metropolitan area, $n = 16$; physician refusal, $n = 8$). Of the remaining 165, 108 were enrolled giving a participation rate of at least 65% (the physician and/or the case could not be identified or contacted for 57 cases, of which the number of cases meeting the inclusion criteria is unknown). Patients included in the case group were similar to those not included for geographic distribution, age ($\leqslant 1$, > 1–5) and hospitalization. Of the eligible patients 33·8% (68/201) were included in the case group during the summer period and 67·7% (40/59) during the 'non-summer period'. Cases included were nationally distributed. All cases had loose stools, 79·6% of whom had a temperature > 38 °C and 35·2% vomiting. Diarrhoea lasted from

Table 1. Salmonella enteritidis, *case-control study, France, 1995: Cases and controls by consumption of chicken and eggs' cooking procedures*

Variables	Cases *n* (%)	Controls *n* (%)	OR*	95% CI (*P* value)
Eating chicken bought ready to eat†	9/87 (10·3)	0/92 (0·0)	Indef.	(0·001)
Eating undercooked chicken	22/75 (29·3)	20/89 (22·5)	1·4	0·7–2·7
Parents eating undercooked chicken	8/101 (7·9)	2/102 (2·0)	4·0	0·9–18·8
Eggs' cooking procedure				
Soft boiled	29/101 (37·2)	21/102 (27·6)	3·0	0·5–30·4
Scrambled + fried‡	28/101 (35·9)	29/102 (38·1)	2·0	0·5–9·1
Hard boiled	21/101 (26·9)	26/102 (34·2)	1·0	Reference
Duration of hard-boiled eggs cooking ⩽ 8 min	15/50 (30·0)	2/43 (4·7)	8·8§	1·7–60·8

* Matched OR.
† Among the children who ate chicken.
‡ Sunny side up.
§ Unmatched.

2 to 21 days (median 8 days). The proportion of cases admitted to hospital was 29·6% (32/108).

Questionnaires were completed for 108 cases and 105 controls (for 3 cases, no controls meeting the matching criteria could be identified). Ninety-three controls were selected among patients of the case physician and 12 from the Minitel telephone directory. The analysis was therefore done on 105 case-control pairs sets. They were 22 and 83 pairs among the age group ⩽ 1 year and 1–5 years, respectively. Cases and controls did not differ in socio-demographic characteristics (rural or urban environment, number of children per household, occupation), day-care or nursery school attendance.

Foods consumption and cooking habits

Forty percent (42/105) of the cases and 40% (42/105) of the controls had meals outside their homes ($P = 0·9$).

Ten percent of cases ate ready to eat chicken compared with none of the controls ($P = 0·001$, Table 1). Eating undercooked chicken was not significantly associated with illness but parents of cases reported eating undercooked chicken four times more often than parents of controls (Table 1).

Although the consumption of undercooked ground beef was not associated with *Salmonella enteritidis* infection among children (matched OR 1·6, 95% CI 0·5–4·9), 43% of parents of cases (34/79) reported eating undercooked ground beef *vs.* 21·1% (16/76) for parents of controls (OR 4·0, 95% CI 1·3–12·0).

Seventy-nine cases (75·2%) and 79 controls (75·2%) ate eggs (OR 1·0, 95% CI 0·5–2·2). Among cases and controls who ate eggs, there was a threefold greater risk of illness (95% CI 0·5–30·4) for eating soft-boiled eggs than for eating hard-boiled eggs (Table 1) although the difference did not reach statistical significance. The risk of illness was strongly associated with a duration of cooking hard-boiled eggs less than 8 min (OR 8·8, 95% CI 1·7–60·8, Table 1). We summarized the exposure to raw, undercooked eggs or food containing raw or lightly cooked eggs as a composite unique variable (Table 2): the risk of illness was twofold (OR 2, 95% CI 1·0–3·8) for children exposed than for children not exposed. When the analysis was done in each age group, a statistically significant increased risk was found only for children > 1 year (OR 2·4, Table 2).

Most case (96/105, 91·4%) and control parents (99/105, 94·3%) reported storing eggs in the refrigerator (OR 1·6, 95% CI 0·5–6·2). The risk for developing *Salmonella enteritidis* infection was almost four times greater (OR 3·8, 95% CI 1·4–10·2) for storage of eggs more than 2 weeks after purchase. This association was stronger (OR 6) during the summer period than during the non-summer period (OR 2·3, Table 2).

We did not find an increased risk of illness associated with purchasing eggs from local rural producers; 29·5% (31/105) of cases and 31·4% (33/105) of controls ate eggs regularly purchased from small local producers (OR 0·9; 95% CI 0·5–1·7).

Other exposures

Cases and controls in both age groups did not differ for prematurity, underlying disease, recent use of

Table 2. Salmonella enteritidis *case-control study, France, 1995. Cases and controls according to cooking procedures and eggs' storage at home*

Variables	Cases *n* (%)	Controls *n* (%)	OR*	95% CI
'Raw, undercooked egg'†	61/104 (58·7)	47/105 (44·8)	2·0	1·0–3·8
⩽ 1 year	3/22 (13·6)	3/22 (13·6)	1·0	0·2–4·9
> 1 to 5 years	59/83 (71·1)	44/83 (53·0)	2·4	1·2–4·8
Length of eggs storage > 2 weeks‡	19/103 (18·4)	5/105 (4·8)	3·8	1·4–10·2
Summer	12/64 (18·7)	2/66 (3·0)	6·0	1·3–26·8
Non-summer	7/39 (17·9)	3/39 (7·7)	2·3	0·6–9·0

* Matched OR.
† We summarized the exposure to raw, undercooked eggs or food containing raw or lightly cooked eggs as a composite unique variable.
‡ Total cases = 103 (2 answers, 'does not know').

Table 3. Salmonella enteritidis, *case-control study, France 1995. Cases and controls by the occurrence of diarrhoea in the household*

Household contacts	Cases *n* (%)	Controls *n* (%)	*P**
Time of onset of diarrhoea			
Before	17 (17·2)	1 (1·0)	0·0004
Same time	28 (28·3)	0 (0·0)	< 0·0001
None	54 (54·5)	102 (99·0)	Reference
Total	99	103	
⩽ 1 year†			
Before	8 (40·0)	0 (0·0)	0·01
Same time	2 (10·0)	0 (0·0)	0·5
None	10 (50·0)	22 (100·0)	Reference
> 1 to 5 years‡			
Before	9 (11·4)	1 (1·2)	0·03
Same time	26 (32·9)	0 (0·0)	< 0·0001
None	44 (55·7)	80 (98·8)	Reference

* Matched *P*, McNemar test.
† Total cases, 20 (2 answers, 'does not know', for the time of the onset), total controls, 22.
‡ Total cases, 79 (2 answers, 'does not know' and 2 answers 'other', for the time of the onset), total controls, 81 (2 answers, 'does not know', for the moment of the occurrence).

Table 4. Salmonella enteritidis, *case control study, France 1995. Multivariate analysis*

	Summer period		Non-summer		Total period	
Variables	OR	95% CI	OR	95% CI	OR	95% CI
'Raw, undercooked egg'*	1·8	0·8–4·0	2·2	0·8–6·7	2·0	1·0–3·8
Storage of eggs > 2 weeks	5·4	1·2–24·4	2·5	0·6–9·9	3·6	1·3–9·8

* We summarized the exposure to raw, undercooked eggs or food containing raw or lightly cooked eggs as a composite unique variable.

medications (antacids, antibiotics in the previous month), immunosuppressive therapy and past or current breast feeding. A total of 42·8% of the cases and 38·1% of the controls had a pet at home ($P = 0·6$). The type of animal did not differ between cases and controls (no case and one control had a turtle).

Person-to-person transmission

Diarrhoea occurred 10–3 days before the onset of symptoms of the case in at least one household member or a close contact of 17 (17·2%) cases and one (1·0%) control ($P = 0{\cdot}0004$, Table 3). Although the OR was undefined, the strength of association appears to be greater for this exposure in the age group $\leqslant 1$ year (8 of 22 cases versus none of 22 controls, $P = 0{\cdot}01$) than in the age group 1–5 years (9 of 79 cases and 1 of 81 controls, $P = 0{\cdot}03$, Table 3). For cases, contacts who had diarrhoea 10–3 days prior to onset of illness were the father or the mother (47·1%), a brother or a sister (35·3%), someone at the place of care (nursery or day-care, 11·8%) or other contacts (5·9%).

Multivariate analysis

The following variables were included: 'Raw, undercooked eggs', duration of storage of eggs, chicken preparation practices for the parents and ground beef cooking procedure of the parents. The multivariate analysis showed, without taking into account the season, that only the consumption of raw or undercooked egg-containing food and the storage of eggs more than 2 weeks after the purchase were associated with *Salmonella enteritidis* infection (Table 4). Storing eggs more than 2 weeks was a statistically significant risk factor of *Salmonella enteritidis* infection for the summer season only (Table 4).

DISCUSSION

This study showed that the consumption of raw, undercooked eggs or food containing raw or lightly cooked eggs (eg homemade mayonnaise, chocolate mousse) is a risk factor for sporadic *Salmonella enteritidis* infection among children $\leqslant 5$ years in France. In addition to raw eggs or undercooked egg products we observed a greater risk of illness for eating soft-boiled eggs than hard-boiled eggs. Although this relation is not statistically significant, this trend is consistent with an experimental study in which *Salmonella enteritidis* was able to survive after 4 min of boiling [5]. In addition, cooking hard-boiled eggs less than 8 min was a strong risk factor.

Our findings also document epidemiologically the hazards of prolonged storage of eggs, particularly during summer, which is also supported by experimental studies [6]. This association may be explained by several reasons: food stores are not obliged to keep eggs refrigerated and one can assume that in summer, the temperature in small groceries increases; the temperature of the refrigerator may not always be correct, particularly in summer in the door's shelves where eggs are usually stored.

In France, cases associated with recognized outbreaks have been found to be associated with eggs either bought from farms or small local producers [2]. In this study we did not confirm this finding. However, matching controls to cases on place of residence could have biased, to some extend, this association towards the nul.

We also found that the consumption of chicken bought ready-to-eat was a risk factor for acquiring *Salmonella enteritidis* infection. Undercooked chicken was not a risk factor if consumed by children $\leqslant 5$ years but was associated with an increased risk for children if eaten by parents. This result may illustrate an information bias; it is also compatible with person-to-person transmission within the household: this may occur if parents get infected from undercooked poultry meat and transmit the infection to their children, particularly infants.

Our study also documents the role of person-to-person transmission which is predominant among infants. Person-to-person transmission of *Salmonella enteritidis* has not been very much implicated in the past, although it has been reported for nosocomial infections [7]. Among children, the predominant mode of transmission differs by age: *Salmonella enteritidis* infection for children $\leqslant 1$ year is mainly related to the exposure to a contaminated person whereas for children > 1–5 years it is related to the consumption of raw or undercooked egg products or chicken.

The participation rate may have affected representativeness. However, patients included in the case group were similar to those not included for geographic distribution, age and hospitalization. Recall bias could have affected our findings, if parents of cases seeking an explanation for their illness were more likely than parents of controls to remember. In addition, the interviewer was not blinded to the case/control status, therefore potentially introducing an information bias. However, the questionnaire had been structured in order to minimize this bias. Food histories were assessed for the month prior to the onset of illness in the case and therefore correspond to food preferences. Because food habits do not vary very much over time among young children, we

chose to interview on food preferences since the responses were expected to be more reliable.

Our results cannot be extrapolated to adults, the main objective was to assess the risk factors among young children who are much more susceptible to salmonella infection than adults. Some of the subgroup analysis were based on a small number of observations resulting in a lack of statistical power. The matched design of our study could have resulted in overmatching for some variables, particularly for the exposure to farm eggs.

Four previous case-control studies have shown a strong association between *Salmonella enteritidis* infection and the consumption of raw or undercooked eggs, two in the UK [8, 9] and two in the US [10, 11]. These studies implicated the role of egg or egg-containing food products, however, they did not explore the influence of the mode of preparation [8, 11], and the role of eating raw shell egg products or runny eggs [9]. A case-control study found that the illness was associated with the extent to which eggs were cooked [10]. However, these studies were targeted at adults.

Poultry meat is an important source of salmonella infections in humans [1]. Chicken has been found to be associated with foodborne disease outbreaks of *Salmonella enteritidis* infections in England and Wales [12]. Chicken bought ready-to-eat has been implicated in an outbreak of *Salmonella typhimurium* infections in France [13], in sporadic cases of *Salmonella enteritidis* infections in Great Britain [9], and more recently as a risk factor for the increase of *Salmonella* Hadar infection observed in France [14]. Furthermore, a British study found that among 20 fresh chilled chicken on retail sale, 8 were contaminated with *Salmonella enteritidis* [15].

Despite the implementation of a national control programme, in France in 1990, the number of *Salmonella enteritidis* isolates has increased until 1995 [1]. Therefore, to limit the occurrence of *Salmonella enteritidis* infection, measures should be discussed at the consumer level. This study indicates that the risk of *Salmonella enteritidis* infection acquired through the consumption of contaminated eggs, egg products and poultry at home may be reduced by limiting the length of storage of eggs and eating eggs and chicken well done. In addition transmission of *Salmonella enteritidis* to young children from a household member or a contact who has been contaminated through the consumption of undercooked egg products or chicken may be of importance.

ACKNOWLEDGEMENTS

The authors thank the General Practitioners who helped to recruit controls and the families who accepted to answer to our questionnaire.

This study was conducted as part of routine activities in the institutions concerned and by an educational grant from the Fondation de France. The Réseau National de Santé Publique is funded by the Ministère de la Santé et de l'assurance Maladie. The Centre National de Référence pour les *Salmonella* et *Shigella* is funded by the Ministère de la Santé et de l'assurance Maladie and the Pasteur Institute.

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