Risk factors of sporadic of *Salmonella* and *Salmonella* typhimurium infections in Castellon (Spain): a matched case-control study

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Summary

Background: The objective of this study was to investigate the risk factors of sporadic *Salmonella* (SA) and *S.* typhimurium (ST) infections to make appropriate recommendations to prevent these diseases.
Methods: From September 2014 to August 2015, a population-based matched (age and gender) case-control (1:2) study was carried out by the epidemiologic division of the Public Health Center of Castellon (Spain). Cases were defined as patients with positive SA and ST cultures that had been isolated from feces and reported by the microbiology laboratories of the Castellon Hospital General and Vila-real Hospital La Plana (Spain). Salmonella serotype was identified in the National Centre of Microbiology (Madrid, Spain).
Results: We reported a total number of 327 SA cases. After excluding SA infections from outbreaks, we studied 212 cases among which 148 where ST infections. A total of 424 matched controls where included in the study. The 49.5% of cases were children <5 years old. Common risk factors associated with SA and ST infections were consumption of pork products and dried pork sausages, contact with diarrhea patients, and mammals at home. Veal and turkey meat consumption was protective. Interestingly, we observed that the higher the intake of pork products, the greater the risk of case's hospitalization.
Conclusions: SA infections mostly affected young children and ST infections were associated with the consumption of pork products.

Key words:
Salmonella Infection. *Salmonella* typhimurium. Case-control. Risk factors. Swine. Child.

Factores de riesgo de infecciones esporádicas por *Salmonella* y *Salmonella* typhimurium en Castellón (España): un estudio caso-control apareado

Resumen

Introducción: El objetivo fue investigar factores de riesgo de infecciones esporádicas por *Salmonella* (SA) y *Salmonella* typhimurium (ST) para realizar recomendaciones preventivas de estas enfermedades.
Métodos: Desde septiembre del 2014 hasta agosto de 2015, la Sección de Epidemiología del Centro de Salud Pública de Castellón llevó a cabo un estudio caso-control apareado (1:2) por edad y género. Los casos fueron pacientes con coprocultivos positivos a SA y ST, notificados por los Laboratorios de Microbiología de los Hospitales General de Castellón y La Plana de Vila-real. El serotipo de las Salmonellas fue identificado en el Centro Nacional de Microbiología (Madrid).
Resultados: Se notificaron 327 casos de infecciones por SA, y se estudiaron 212 casos, de ellos fueron 148 infecciones por ST y 424 controles. Se excluyeron infecciones SA ocurridas en brotes. El 49,5% de los casos eran niños menores de 5 años. Los factores de riesgos fueron consumo de productos porcinos, longanizas secas de cerdo, contacto con pacientes con diarrea, y presencia de algún mamífero en el hogar. El consumo de ternera y pavo fue protector. Se observó más riesgo de hospitalización a mayor consumo de productos porcinos.
Conclusiones: Las infecciones por SA afectaron principalmente a niños pequeños, y las infecciones por ST se asociaron al consumo de productos porcinos.

Palabras clave:
Salmonella Infección. *Salmonella* typhimurium. Caso-control. Factores riesgo. Cerdo. Niños.

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Introduction

Salmonella typhimurium (ST) and *Salmonella* Enteritidis (SE) are the most frequent etiologic agents of *Salmonella* infections (SA) in some countries[1], including Spain. In this country, ST has a higher incidence than SE[2] and the cost of these infections to society is high[3]. While SE infections and foodborne outbreaks have been well documented and frequently related to chicken eggs consumption[4], ST serotype has a great variety of animal reservoirs and it is commonly presented as sporadic cases where the source of infection is usually unknown[5]. However, recent studies indicate that the main risk factor of ST and its monophasic variant in Europe is pork products consumption[6].

In Spain, investigations into recent foodborne outbreaks caused by monophasic ST found that the consumption of pork products was associated with the infection[7-9]. Nevertheless, few studies have been conducted on sporadic salmonellosis to assess the source of the infection[10].

The objective of this study was to find the factors associated with the incidence of sporadic SA and ST infections in Castellon to propose appropriate control and prevention measures.

Materials and methods

A population-based matched case-control study was carried out by the Epidemiology Division (ED) of the Public Health Center in Castellon (Spain) from September 2014 to August 2015. The cases were positive *Salmonella* culture isolated from feces by the Microbiology Laboratories of Hospital General of Castellon (HGC) and Hospital La Plana-Vila-real (HPV) and serotyped at the National Centre of Microbiology (NCM) at Majadahonda (Madrid). SA patients associated with outbreaks were excluded, as were those isolated from blood, urine, and skin abscesses. For each case, two controls (1:2) matched by age (one year) and gender were obtained. Two age groups were considered for ST, 0-4 years old and 5 years and over.

When the ED received a positive *Salmonella* culture, the physician, who had attended the patient and requested the *Salmonella* culture, was contacted and permission was sought to interview two controls from their patient list. Two controls and two other reserve controls were randomly chosen, matched by age and gender. ED staff interviewed the *Salmonella* patients and the controls by telephone using the same questionnaire. If the controls had had from diarrhea in the previous 30 days or lived in a nursing home, they were excluded.

The questionnaire covered several potential factors associated with SA, including contact with patients who have diarrhea, taking antibiotics in the last 15 days, habitually consuming of certain foods (meat, fish, eggs), drinking unsafe water, food hygiene practices at home, animals at home, attending day-care or school, restaurant visit in the last 15 days, and some demographic questions. Food consumption was collected as the number of times per week. Food consumed less often than once a week was considered not consumed.

Microbiology methods

Stool samples were inoculated into the usual culture media. *Salmonella* isolates were serotyped in the microbiology laboratories of HGC and HPV by agglutination on a slide according to the Kauffmann-White scheme, using polyvalent and monovalent antisera to somatic and to flagellar antigens (Bio-Rad Statents Serum Institute). In the HPV, strains where all characterized as *Salmonella* spp. The following serotypes: ST, SE, *S.* Typhi*, S.* Hadar and *S.* Virchow were identified in the HGC. The rest of Salmonella strains were only characterized as group B, C and D or as *Salmonella* spp. Every *Salmonella* strain was referred to the NCM for its complete serotyping and phage typing. More detailed information of microbiology methods is presented in the case-case study of Arnedo-Pena and co-authors[11].

Statistical methods

Cases and controls where compared using several tests including Chi^2 and Fisher's exact, median non-parametric and Kruskal-Wallis. The mean and standard deviation was calculated for each food consumed. In addition, a test for trends of the odds was used to estimate a dose-response relationship between food consumption and cases versus controls. Bivariate and multivariate conditional logistic regression was used to analyses cases and controls and matched odds ratio (mOR) as measures of association with a 95% confidence interval (CI) were calculated. In the multivariate analyses, only factors associated with a $p<0.10$ in the conditional logistic regression were included in the first model. After, models were running with removing one by one co-variables $p<0.05$ until a model was obtained with all co-variables having a $p<0.05$. Automatic stepwise methodology was not used. Two multivariate models were made considering the co-variables dried pork sausages and all pork products consumption. An etiologic fraction was estimated from the matched case-control study[12] with three levels of food consumption exposure (no consumption, once per week, more than once per week) and two age groups, 0-4 years old, and 5 years and over. Multinomial logistic regression was used to compare the factors studied and patient's hospitalization; a relative risk ratio (RRR) with 95% CI was

estimated. Considering 300 patients with positive *Salmonella* culture by year in Castellon, a power of 78% may be achieved to detect an OR of 1.50, with 60% exposure of controls, α=0.05, and a matched case-control design. For positive ST culture and 200 patients, the power could be 60% with similar conditions. The Stata® 14 version 1.0 was used in the statistical analyses.

The Ethics Committee of the Hospital General of Castellon approved the study and verbal consent was obtained from the participants or their parents.

Results

During the study period, 327 SA infections were notified. Positive *Salmonella* culture from feces accounted for 212 patients, whereas positive *Salmonella* culture other than feces (blood, skin, abscesses) was found in 9 patients. SA infections from outbreaks totaled 70 patients. Thus, 291 patients were considered from the SA notified, 89.0% (291/327).

Our study included 212 SA cases with positive culture and 424 matched controls. Controls participation was 88.9% (424/477). To obtain these controls, 772 telephone consultations were made. The lags between the date of *Salmonella* notifications and the interviews of the cases and their controls had of mean 61.1 ±38.9 days and 70.9±39.4 days, respectively.

Table 1 shows the characteristics of patients with SA infections and the controls. The mean age of the cases was 15.3±23.2 years with a median of 5.0 years, and a range of 0.01-91 years. The 49.5% of cases were children less 5 years old. In accordance with the matched approach, age and gender had a similar distribution among two groups. No significant differences were observed in terms of country of origin, residence, or infection onset by season. ST infections represented 69.8% (148/212) of cases and SE infections 8.5% (18/212).

Table 2 presents the results of the matched case-control comparing SA patients and controls. Risk factors associated with a significant increase of SA infections included Spanish origin, consumption of dried pork sausages and pork products, dog, cat, or other mammals at home, taking antibiotic in the last 15 days, and contact with diarrhea patients. Protective factors were consumption of turkey or veal, consumption of other foods with eggs, and attending day care or school. Considering the multivariate analyses model 1, SA infections were associated with the consumption of dried pork sausages (mOR=1.29; 95% CI 1.08-1.53), a 29% increase of SA infections per each day of dried pork consumption. Dog, cat, or other mammals at home, taking antibiotics, and contact with diarrhea patients were associated with SA infections with mOR=1.57 (95% CI 1.05-2.35), mOR=1.73 (95% CI 1.06-2.83), and mOR=3.68 (95% CI 1.77-7.64), respectively. Turkey and veal meat intake were protective factors. In the multivariate model 2, SA infections were associated with the consumption of pork products (mOR=1.14, 95% CI 1.05-1.25). The association of other factors remained similar, except for dog, cat, or other mammals at home, which was not significant. The median and trend tests estimated that SA infections rose with increased when consumption of dried pork sausages and pork products, and the consumption of turkey, veal, and other foods with eggs had a protective effect.

In the bivariate analyses (Table 3) ST infections were associated with Spanish origin, consumption of dried pork sausages, pork products, chicken egg omelets, dog, cat, or other mammals at home, and contact with diarrhea patients. Protective factors were Maghreb origin, consumption of turkey, veal, or fish, consumption of other foods with egg, and attending day care or school. In the multivariate analyses model 1, dried pork sausages consumption (mOR=1.33; 95% CI 1.08.1.63), chicken egg omelets (mOR=1.27;

Table 1. Characteristics of sporadic positive *Salmonella* culture patients and the controls.

Variables	*Salmonella* all serotypes n=212 N (%)	Controls n=424 N (%)	*p*-value
Age (years) mean ±SD	15.3±23.2	15.7±23.1	0.291
Age (years) median-range	5.0 (0.01-91)	5.0 (0.01-91)	0.693
Age groups			
<1 year	13 (6.1)	16 (3.8)	
1-4 years	92 (43.4)	183 (43.2)	
5-9 years	40 (18.9)	85 (20.0)	
10-14 years	18 (8.5)	38 (9.0)	
15-34 years	11 (5.2)	26 (6.1)	
35-64 years	20 (9.4)	40 (9.4)	
65 years and more	18 (8.5)	36 (8.5)	0.497
Gender: female	113 (53.3)	227 (53.5)	0.551
Country of origin			
Spain	194 (91.5)	363 (85.6)	
Romania	8 (3.8)	27 (6.4)	
Maghreb	5 (2.4)	21 (5.0)	
Other	5 (2.4)	13 (3.1)	0.093
Residence in Castellon department	119 (56.1)	239 (56.4)	0.995
Infection onset summer season	77 (36.3)	186 (43.9)	0.068
Salmonella serotype			
S. typhimurium	148 (69.8)		
S. Enteritidis	18 (8.5)		
Other *Salmonella* serotypes*	42 (19.8)		
Salmonella spp	4 (1.9)		

*Derby, Rissen, Hadar, Newport, Mikawashima, Virchow,…

Table 2. Comparison of positive *Salmonella* culture patients of all serotypes and controls. Bivariate and multivariate conditional logistic regression.

Variables	Cases N=212 N (%)	Controls N=424 N (%)	Bivariate Analysis mOR[1](95%CI[2])	Multivariate analysis model 1 mOR[I] (95%C[III])	Multivariate analysis model 2 mOR[I] (95%C[III])
Country of origin					
Spain	194 (91.5)	363 (85.6)	1.90 (1.06-3.39)		
Rumania	8 (3.8)	27 (6.4)	0.52 (0.22-1.26)		
Maghreb	5 (2.4)	21 (5.0)	0.46 (0.17-1.25)		
Food consumption, frequency times per week: Mean±SD[III]					
Chicken	2.6±1.3	2.4±1.2	1.09 (0.94-1.26)		
Turkey	0.5±0.9	0.7±1.0*[VI] -	0.80 (067-0.96)	0.77 (0.63-0.94)	0.79 (0.64-0.96)
Veal	0.8±0.8	1.0±1.0*[VI] -	0.73 (0.60-0.90)	0.77 (0.61-0.96)	0.77 (0.61-0.96)
Lamb	0.4±0.6	0.5±0.7	0.80 (0.62-1.03)		
-Pork meat	1.3±1.0	1.2±1.0	1.08 (0.90-1.30)		
-Pork sausages	0.9±0.8	0.8±0.9	1.07 (0.88-1.29)		
-Cold pork meats	2.0±1.9	1.7±2.0	1.05 (0.95-1.15)		
-Dried pork sausages	0.8±1.4	0.5±0.9[VI] +	1.29 (1.10-1.50)	1.29 (1.08-1.53)	NI[IV]
-Pork products[V]	4.0±2.4	3.5±2.6[VI] +	1.12 (1.03-1.21)	NI[IV]	1.14 (1.05-1.25)
-Minced meat	1.0±0.8	1.1±0.9	0.92 (0.75-1.14)		
-Fish	2.1±1.5	2.4±1.5*[VI] -	0.87 (0.77-0.98)		
-Chicken egg mayonnaise	0.3±0.8	0.3±0.8	1.02 (0.81-1.27)		
-Chicken egg omelets	1.6±1.1	1.6±1.0	1.04 (0.88-1.23)		
-Other foods with eggs	0.3±0.9	0.5±1.1*[VI] -	0.73 (0.60-0.90)	0.72 (0.57-0.90)	0.71 (0.57-0.90)
Drinking unsafe water	30 (14.2)	50 (11.8)	1.29 (0.76-2.19)		
Raw meat in home kitchen	179 (84.4)	355 (83.7)	1.08 (0.63-1.85)		
Handling raw meat	83 (18.4)	164 (38.7)	1.02 (0.72-1.44)		
Restaurant visit 15 days before onset	76 (35.8)	163 (38.4)	0.86 (0.57-1.28)		
Attending day-care/school	95 (44.8)	252 (59.4)	0.33 (0.20-0.53)	0.29 (0.17-0.49)	0.27 (0.16-0.45)
Playing in soil	94 (44.3)	180 (42.5)	1.13 (0.74-1.72)		
Play in places with pigeons	60 (28.3)	113 (26.7)	1.10 (0.74-1.65)		
Animals at home	111 (52.4)	188 (44.3)	1.38 (0.99-1.94)		
-Dog, cat, other mammals at home	86 (40.6)	137 (32.3)	1.48 (1.03-2.11)	1.57 (1.05-2.35)	
-Birds	40 (18.9)	56 (13.2)	1.55 (0.99-2.44)		
Reptiles	8 (3.8)	18 (4.2)	0.88 (0.38-2.07)		
Taking antibiotics in the last 15 days	45 (21.2)	58 (13.7)	1.70 (1.10-2.61)	1.73 (1.06-2.83)	1.79 (1.09-2.93)
Contact with diarrhea patients	26 (12.3)	16 (3.8)	3.53 (1.83-6.78)	3.68 (1.77-7.64)	3.54 (1.71-7.32)

[I]mOR: matched odds ratio; [II]CI: confidence interval; [III]Standard deviation; [IV]Not included; [V]Pork products include pork meat, pork sausages, cold pork meats, and dried pork sausages *Median test p<0.05, [VI]Chi^2 test for trends of odds p<0.05 (-) protector (+) risk.

95% CI 1.01-1.59), dogs, cats, or other mammals at home (mOR=2.04; 95% CI 1.23-3.41), and contact with diarrhea patients (mOR=4.25; 95% CI 1.79-10.11) were significant risk factors; and the protective factors were Maghreb origin, consumption of turkey, veal, other foods with eggs, and attending day care or school. In the multivariate analyses model 2, ST infections were associated with pork products consumption (mOR=1.19; 95% CI 1.07-1.32), dog, cat, and other mammals at home (mOR=1.94;1.17-3.19), and contact with diarrhea patients (mOR=3.73; 95% CI 1.59-8.74). Protective factors were consumption of turkey, veal, other foods with eggs, and attending day care or school. The median and trend tests estimated an increase of ST infections with increased consumption of dried pork sausage and pork products. In addition, the consumption of veal, fish and other foods with eggs were protective factors. Etiologic fraction estimated that 61.1% of the ST infections may be attributable to the consumption of pork products.

The matched case-control study of ST patients included two age groups, 0-4 years old and 5 years and over. In the 0-4 years old group (Table 4), salmonellosis was associated with the consumption of dried pork sausages, chicken egg mayonnaise, and taking antibiotics; the protective factors were consumption of veal, and other foods with eggs. In the multivariate analyses model 1, the

Table 3. Comparison of positive *S.*typhimurium culture patients and controls. Bivariate and multivariate conditional logistic regression.

Variables	Cases N=148 N (%)	Controls N=296 N (%)	Bivariate Analysis mOR[1](95%CI[2])	Multivariate analysis model 1 mOR[I] (95%C[III])	Multivariate analysis model 2 mOR[I] (95%C[III])
Country of origin:					
Spain	135 (91.2)	247 (83.4)	2.20 (1.11-4.38)		
Rumania	8 (5.4)	25 (8.4)	0.57 (0.23-1.39)		
Maghreb	2 (1.4)	15 (5.1)	0.27 (0.06-1.17)	0.18 (0.03-0.91)	
Food consumption frequency times per week: Mean±SD[III]					
Chicken	2.6±1.3	2.6±1.2	1.04 (0.87-1.23)		
Turkey	0.6±0.9	0.7±1.0*	0.83 (0.67-1.03)	0.75 (0.57-0.97)	0.78 (0.60-0.99)
Veal	0.8±0.8	1.1±1.0*[VI] -	0.70 (0.55-0.90)	0.74 (0.57-0.96)	0.73 (0.56-0.94)
Lamb	0.4±0.6	0.5±0.7	0.91 (0.68-1.22)		
-Pork meat	1.4±1.0	1.3±1.0	1.07 (0.86-1.32)		
-Pork sausages	0.9±0.8	0.8±0.9	1.08 (0.86-1.36)		
-Cold pork meats	2.0±1.9	1.7±2.0	1.09 (0.97-1.22)		
-Dried pork sausages	0.9±1.4	0.5±1.0*[VI] +	1.36 (1.14-1.62)	1.33 (1.08-1.63)	NI[4]
-Pork products[V]	4.4±2.3	3.7±2.5*[VI] +	1.18 (1.07-1.30)	NI[4]	1.19 (1.07-1.32)
-Minced meat	1.1±0.8	1.2±1.0	0.96 (0.76-1.21)		
-Fish	2.1±1.5	2.5±1.5*[VI] -	0.84 (0.72-0.98)		
-Chicken egg mayonnaise	0.4±0.9	0.3±0.8	1.13 (0.87-1.46)		
-Chicken egg omelets	1.8±1.2	1.6±1.0*	1.18 (0.98-1.43)	1.27 (1.01-1.59)	
-Other foods with eggs	0.3±0.9	0.6±1.2*[VI] -	0.74 (0.58-0.94)	0.74 (0.56-0.97)	0.72 (0.54-0.95)
Drinking unsafe water	19 (12.8)	28 (9.5)	1.55 (0.77-3.12)		
Raw meat in home kitchen	56 (37.8)	112 (37.8)	0.97 (0.53-1.78)		
Handling raw meat	121(81.8)	243 (82.1)	1.00 (0.66-1.52)		
Restaurant visit 15 days before onset	59 (40.0)	124 (41.9)	0.90 (0.57-1.42)		
Attending day-care/school	76 (51.4)	192 (64.9)	0.40 (0.23-0.67)	0.36 (0.20-0.67)	0.37 (0.21-0.65)
Playing in soil	77 (52.0)	145 (49.0)	1.20 (0.74-1.97)		
Play in places with pigeons	42 (28.4)	85 (28.7)	0.98 (0.61-1.57)		
Animals at home	77 (52.0)	128 (43.2)	1.47 (0.97-2.24)		
-Dog, cat, other mammals at home	60 (40.5)	89 (30.1)	1.69 (1.09-2.64)	2.04 (1.23-3.41)	1.94 (1.17-3.19)
-Birds at home	27 (18.2)	36 (12.2)	1.65 (0.94-2.88)		
Reptiles at home	5 (3.4)	12 (4.1)	0.83 (0.29-2.37)		
Taking antibiotics in the last 15 days	30 (20.3)	39 (13.2)	1.66 (0.99-2.80)		
Contact with diarrhea patients	19 (12.8)	12 (4.1)	3.54 (1.63-7.65)	4.25 (1.79-10.11)	3.73 (1.59-8.74)

[I]mOR: matched odds ratio; [II]CI: confidence interval; [III]Standard deviation; [IV]Not included; [V]Pork products include pork meat, pork sausages, cold pork meats, and dried pork sausages *Median test p<0.05, [VI]Chi^2 test for trends of odds p<0.05 (-) protector (+) risk.

consumption of dried pork sausages (mOR=2.07; 95% CI 1.46-2.93), and other foods with eggs were associated factors; taking antibiotics showed a marginal association. In the multivariate analyses model 2, the consumption of pork products (mOR=1.26; 95% CI 1.09-1.45) and chicken eggs mayonnaise (mOR=1.80; 95% CI 1.07-3.04) were risk factors; consumption of other foods with eggs was a protective factor. Increased the consumption of dried pork sausages, pork products and chicken egg mayonnaise resulted in more ST infections, whereas an increased consumption of veal or other foods with eggs reduced the number of these infections.

For the 5 years and over group (Table 5) in the bivariate analyses, ST infections were associated with consumption of chicken egg omelets, animals at home, and contact with diarrhea patients; consumption of fish and attending day care or school were protective factors. In the multivariate analyses, chicken omelets consumption (mOR=1.62; 95% CI 1.21-2.36) and contact with diarrhea patients (mOR=12.27; 95% CI 1.59-70.17) were risk factors and attending day care or school was a protective factor. The trends test estimated that an increase in the consumption of chicken egg omelets increased ST infections.

The bivariate multinomial analyses ST infections considered three groups: controls, patients without hospital admission, and patients with hospital admission (Table 6). The consumption of dried pork sausages, pork products, dog, cat,

Table 4. Comparison of positive *S.*typhimurium culture patients and controls in the 0-4 years old group. Bivariate and multivariate conditional logistic regression.

Variables	Cases N=80 N (%)	Controls N=160 N (%)	Bivariate Analysis mOR[1](95%CI[2])	Multivariate analysis model 1 mOR[I] (95%C[III])	Multivariate analysis model 2 mOR[I] (95%CI[II])
Country of origin					
Spain	71 (88.8)	129 (80.7)	2.08 (0.87-4.97)		
Rumania	7 (8.8)	17 (10.6)	0.91 (0.32-2.60)		
Maghreb	2 (2.5)	12 (7.5)	0.33 (0.07-1.49)		
Food consumption frequency times per week: Mean±SD[III]					
Chicken	2.6±1.2	2.6±1.2	0.98 (0.78-1.23)		
Turkey	0.6±0.9	0.7±1.0	0.89 (0.67-1.18)		
Veal	0.9±0.8	1.2±1.1*[VI] -	0.71 (0.52-0.97)		
Lamb	0.4±0.6	0.5±0.7	0.75 (0.48-1.17)		
-Pork meat	1.2±0.9	1.2±1.0	1.05 (0.79-1.41)		
-Pork sausages	0.9±0.8	0.8±0.9	1.06 (0.77-1.46)		
-Cold pork meats	1.8±1.9	1.4±1.9	1.13 (0.96-1.38)		
-Dried pork sausages	1.1±1.5	0.4±0.9*[VI] +	2.03 (1.44-2.85)	2.07 (1.46-2.93)	NI[IV]
-Pork products[V]	4.4±2.4	3.4±2.4*[VI] +	1.25 (1.09-1.44)	NI[IV]	1.26 (1.09-1.45)
-Minced meat	1.2±0.9	1.1±1.0	1.06 (0.77-1.45)		
-Fish	2.2±1.5	2.5±1.5	0.89 (0.73-1.08)		
-Chicken egg mayonnaise	0.3±0.8	0.1±0.5[VI] +	1.63 (1.03-2.55)		1.80 (1.07-3.04)
-Chicken egg omelets	1.6±1.2	1.6±1.0	1.01 (0.78-1.32)		
-Other foods with eggs	0.2±0.6	0.5±1.0*[VI] -	0.59 (0.39-0.89)	0.60 (0.37-0.95)	0.52 (0.32-0.82)
Drinking unsafe water	7 (8,8)	14 (8.8)	1.00 (0.34-2.93)		
Raw meat in home kitchen	63 (78.8)	127 (79.4)	0.95 (0.43-2.08)		
Handling raw meat	27 (33.8)	60 (37.5)	0.82 (0.44-1.53)		
Restaurant visit 15 days before onset	30 (37.5)	60 (37.5)	1.00 (0.52-1.93)		
Attending day-care/school	49 (61.3)	106 (66.3)	0.75 (0.40-1.43)		
Playing in soil	53 (66.3)	106 (66.3)	1.00 (0.53-1.90)		
Play in places with pigeons	29 (36.3)	63 (39.4)	0.87 (0.50-1.53)		
Animals at home	39 (48.8)	72 (45.0)	1.18 (0.67-2.08)		
-Dog, cat, other mammals at home	31 (38.8)	46 (28.8)	1.65 (0.91-3.03)		
-Birds at home	15 (18.8)	24 (15.0)	1.34 (0.64-2.82)		
Reptiles at home	3 (3.8)	5 (3.1)	1.20 (0.29-5.02)		
Taking antibiotics in the last 15 days	20 (25)	19 (11.9)	2.45 (1.22-4.93)	1.98 (0.99-4.82)	
Contact with diarrhea patients	9 (11.3)	9 (5.6)	2.10 (0.80-5.49)		

[I]mOR: matched odds ratio: [II]CI: confidence interval; [III]SD: Standard deviation; [IV]Not included; [V]Pork products include pork meat, pork sausages, cold pork meats, and dried pork sausages; *Median test p<0.05, [VI]Chi[2] Test for trends of odds p<0.05 (-) protector (+) risk.

or other mammals at home, and contact with diarrhea patients raised ST infections. Consumptions of veal, other foods with eggs, and attending day care or school were protective factors. A 29% increase of ST infections per each day of dried pork sausages consumption was found in patients with hospital admission, and 45% increase in patients admitted to hospital. We observed that the risk of case's hospitalization increased with pork products consumption.

Discussion

This study suggests several groups of factors associated with ST and SA infections. The first group includes risk factors such as consumption of pork products and dried pork sausages. The second group comprises known risk factors such as taking antibiotics in the previous 15 days[10,13], contact with diarrhea patients[14], and dogs, cats, or other mammals at home[10,15]. The third group includes consump-

Table 5. Comparison of positive *S.* typhimurium culture patients and controls in the 5 years and over group. Bivariate and multivariate conditional logistic regression.

Variables	Cases N=68 N (%)	Controls N=136 N (%)	Bivariate Analysis mOR[1](95%CI[2])	Multivariate analysis model 1 mOR[I] (95%CI[II])
Country of origin				
Spain	64 (94.1)	118 (86.8)	2.41 (0.79-7.39)	
Rumania	1 (1.5)	10 (7.4)	0.17 (0.02-1.46)	
Maghreb	0 (0)	3 (2.2)	0.51 (0.0-4.84)	
Food consumption frequency times per week: Mean±SD[III]				
-Chicken	2.6±1.3	2.7±1.1	1.12 (0.87-1.44)	
-Turkey	0.5±0.9	0.8±0.9*	0.76 (0.54-1.07)	
-Veal	0.6±0.7	0.9±0.8	0.69 (0.46-1.03)	
-Lamb	0.5±0.7	0.5±0.7	1.09 (0.72-1.65)	
-Pork meat	1.4±1.0	1.4±0.9	1.08 (0.79-1.49)	
-Pork sausages	0.9±0.9	0.8±0.9	1.10 (0.79-1.55)	
-Cold pork meats	2.2±1.9	2.0±2.1	1.05 (0.90-1.22)	
-Dried pork sausages	0.7±1.3	0.6±1.1	1.04 (0.82-1.31)	
-Pork products[IV]	4.5±2.2	4.1±2.7	1.10 (0.96-1.26)	
-Minced meat	1.0±0.8	1.2±1.0	0.84 (0.58-1.21)	
-Fish	2.0±1.6	2.4±1.4*	0.78 (0.61-0.99)	
-Chicken egg mayonnaise	0.4±1.1	0.5±1.0	0.92 (0.66-1.28)	
-Chicken egg omelets	1.9±1.2	1.5±1.0[V+]	1.43 (1.06-1.92)	1.62 (1.11-2.36)
-Other foods with eggs	0.4±1.1	0.5±1.2	0.87 (0.66-1.17)	
Drinking unsafe water	12 (17.6)	14 (10.3)	2.19 (0.85-5.65)	
Raw meat in home kitchen	58 (85.3)	116 (85.3)	1.00 (0.38-2.66)	
Handling raw meat	29 (42.6)	52 (38.2)	1.18 (0.67-2.07)	
Restaurant visit 15 days before onset	29 (42.6)	64 (47.1)	0.81 (0.42-1.54)	
Attending day-care/school	27 (39.7)	86 (63.2)	0.09 (0.03-0.30)	0.09 (0.02-0.30)
Playing in soil	24 (35.3)	39 (28.7)	1.55 (0.73-3.29)	
Play in places with pigeons	13 (19.1)	22 (16.2)	1.28 (0.55-2.97)	
Animals at home	38 (55.9)	56 (41.2)	1.90 (1.02-3.53)	
-Dog, cat, other mammals at home	29 (42.6)	43 (31.6)	1.75 (0.90-3.37)	
-Birds at home	12 (17.6)	12 (8.8)	2.16 (0.92-5.05)	
Reptiles at home	2 (2.9)	7 (5.1)	0.57 (0.12-2.75)	
Taking antibiotics in the last 15 days	10 (14.7)	20 (14.7)	1.00 (0.44-2.26)	
Contact with diarrhea patients	10 (14.7)	3 (2.2)	9.29 (2.02-42.68)	12.27 (1.59-70.17)

[I]mOR: matched Odds ratio: [II]CI: confidence interval; [III]SD: Standard deviation; [IV]Pork products include pork meat, pork sausages, cold pork meats, and dried pork sausages; *Median test p<0.05, [V]Chi[2] Test for trends of odds p<0.05 (-) protector (+) risk

tion of chicken egg mayonnaise and chicken egg omelets[16]. Finally, the fourth group covers protective factors such as consumption of turkey, veal, other foods with chicken eggs, attending at day care or school, and Maghreb origin. In the 5 years and over group, ST infections were not associated with consumption of pork products, but the consumption of chicken egg omelets was a risk factor.

Pork products consumption should be highlighted as a risk factor of ST and SA infections, considering that these foods are usually ready-to-eat and consumed raw. The trend of this consumption suggests a dose-response relationship with these infections and the multinomial analyses also suggests high consumption is linked to an increase in patient hospitalization. One interesting point is the protective factor of Maghreb origin, which may be explained by the prohibition of pork consumption by Muslim religion that is practiced in these countries.

The 0-4 years old group was the most affected by this consumption. In a developed socio-economic context, several factors may be associated with high susceptibility of young children[17], in-

Table 6. Risk factors of positive S.typhimurium culture patients considering hospital admission patients, patients without hospital admission and controls by bivariate multinomial logistic regression analysis.

Variables	Controls N=296	Patients without hospital admission N=124	Patients without hospital admission N=24	P value
Food consumption per week	Reference	RRR (95% CI)	RRR (95% CI)	
Chicken	1.0	1.03 (0.87-1.23)	1.07 (0.23-0.64)	0.880
Turkey	1.0	0.85 (0.68-1.07)	0.69 (0.41-1.19)	0.171
Veal	1.0	0.74 (0.58-0.95)	0.59 (0.34-1.02)	0.011
Lamb	1.0	0.94 (0.69-1.28)	0.75 (0.38-1.48)	0.668
-Pork meat	1.0	1.08 (0.88-1.33)	0.94 (0.61-1.44)	0.707
-Pork sausages	1.0	1.02 (0.80-1.29)	1.35 (0.90-2.04)	0.379
-Cold pork meats	1.0	1.07 (0.96-1.18)	1.07 (0.87-1.31)	0.457
-Dried pork sausages	1.0	1.29 (1.09-1.54)	1.45 (1.10-1.92)	0.003
-Pork products*	1.0	1.11 (1.02-1.22)	1.20 (1.00-1.44)	0.010
-Minced meat	1.0	0.96 (0.77-1.21)	0.97 (0.61-1.53)	0.947
-Fish	1.0	0.84 (0.72-0.97)	0.96 (0.73-1.27)	0.060
-Chicken egg mayonnaise	1.0	1.13 (0.90-1.43)	0.90 (0.49-1.64)	0.520
-Chicken egg omelets	1.0	1.21 (0.99-1.56)	0.93 (0.61-1.41)	0.124
-Other foods with eggs	1.0	0.76 (0.59-0.99)	0.64 (0.33-1.24)	0.033
Drinking unsafe water	1.0	1.32 (0.68-2.56)	1.91 (0.61-6.00)	0.470
Raw meat in home kitchen	1.0	1.13 (0.65-1.99)	0.53 (0.21-1.34)	0.353
Handling raw meat	1.0	0.97 (0.63-1.49)	1.17 (0.50-2.73)	0.915
Restaurant visit 15 days before onset	1.0	0.88 (0.33-1.35)	1.17 (0.51-2.71)	0.745
Attending day-care/school	1.0	0.58 (0.38-0.88)	0.54 (0.24-1.25)	0.024
Playing in soil	1.0	1.08 (0.71-1.64)	1.46 (0.62-3.39)	0.662
Play in places with pigeons	1.0	0.98 (0.61-1.55)	1.02 (0.41-2.55)	0.993
Animals at home	1.0	1.31 (0.86-2.00)	2.19 (0.93-5.16)	0.115
-Dog, cat, other mammals at home	1.0	1.42 (0.91-2.20)	2.75 (1.19-6.37)	0.031
-Birds at home	1.0	1.56 (0.87-2.78)	1.90 (0.67-5.40)	0.220
Reptiles at home	1.0	0.79 (0.25-2.50)	1.03 (0.13-8.27)	0.916
Taking antibiotics in the last 15 days	1.0	1.50 (0.85-2.64)	2.71 (1.06-6.96)	0.084
Contact with diarrhea patients	1.0	3.76 (1.74-8.13)	2.15 (0.45-10.22)	0.003

RRR: relative risk ratio; CI: confidence interval; *Pork products: pork meat, pork sausages, cold pork meats, and dried pork sausages.

cluding immature immunity, gastric hypoacidity, food preferences, and young children' behavior related to the exposure[18]. In addition, due to their low body mass, what are probably low concentrations of *Salmonella* in pork products could cause a severe infection in a young child and more limited or not infection in an adult.

These results are in line with other studies in which pigs were the prime source of human salmonellosis in Italy[19] and Germany[5,20], where the consumption of raw ground pork and uncooked pork sausage were associated with ST and SA infections in children and adults. In the 2008-2015 period, Germany and Spain, the first and second producers of pig meat in the European Community (EC), consumed a mean of pig meat of 54.5 kg/year and 52.2 kg/year per capita respectively, versus a mean of 40.7 kg/year per capita in the EC[21-22].

In this study, the pork product with the highest mOR was dried ready-to-eat sausage that is consumed raw. An outbreak of ST and S. Derby caused by consuming these sausages was reported in Castellon in 2011[8], and case-case study of SA in this population found the same association[11]. In addition, several veterinary investigations have found high prevalence of *Salmonella* in pig farms[23-26] with a range of 33%-73.4%, the pig slaughtering process (10.9%)[27], and some pork products such as meat for dry-cured sausages (13.6%)[28].

In Spain ST infections can be transmitted by egg consumption[16]. In our study consumption of egg mayonnaise was associated with ST infections in the 0-4 years old group and consumptions of egg omelets was associated with ST infections in the 5 years and over group. Mayonnaise is made with raw eggs whereas

omelets are cooked, but it is likely that temperatures were not high enough to remove the risk. No dose-response was found for this consumption but a case-case study of ST infections in this population found the same association[11]. Other case-control studies had found this association in children[20].

Risk factors of ST and SA infections such as taking antibiotics and contact with diarrhea patients are documented[10,13,14] and confirmed in our study. However, contact with diarrhea patients was associated with the total studied and not in the 0-4 years old group, and which taking antibiotics was a risk factor. Dogs, cats, and other mammals at home was a risk factor for ST and SA infections; this risk factor increases of patient's hospitalization. A study has previously found this risk[29].

Protective factors of ST and SA infections were consumption of turkey, veal, and other foods with chicken eggs, and attending day care or school. These results suggest that the other meats such as turkey or veal present lower *Salmonella* concentration and are cooked before consumption. Other foods with chicken eggs include custard, crème caramel, or cake, all of which are consumed cooked. Attending day care or school as a protective factor suggests that sporadic SA infections are not associated with day care or school kitchens.

Some indicated risk factors of SA infections such as improper handling food in the kitchen[30], drinking unsafe water, restaurant visit 15 days before onset of infection[16], birds or reptiles at home[15] and playing in soil were not associated with SA in the study. Exposure to these factors could be too low to be detected with this design. However, a case-case study of this population detected playing in soil as significant risk factor of ST[11]; difference of age among ST patients, higher incidence in children, and presence of other *Salmonellas* serotypes may be explanatory factors. In addition, some studies of kitchen hygiene in of *Salmonella* patients found not differences from controls[31].

This study has several strengths, including the high sample size of *Salmonella* patients with high proportions of *Salmonella* serotyped, the high participation rate of cases and controls, probably motivate by the cooperation of physicians with the study, the sufficient power, and the use of conditional logistic regression.

The limitations of the study include: first, the lags between the date of *Salmonella* notifications and the interviews of cases and their controls were high, but interview questions concerned habitual way food consumptions and some habits and health situations. Second, some well-known risk factors of salmonellosis were not included such as foreign travels or chronic diseases. Third, the power of study was high for SA and ST infections, but it was reduced when age-groups were analyzed. Fourth, some information bias may have been occurred, considering that cases' food consumption could have been recorded more accurately in relation to the disease. Fifth, the quantification of food consumptions may be some imprecise in terms of frequency per week.

Some recommendations in relation to prevent ST and SA infections can be drawn from the study: first, public health education on the risk of pork products consumption should be improved. Second, swine production from farm to table should be ameliorated with a national program of control and surveillance. Third, egg production needs tighter control to lower the risk of SA infections. Fourth, a dedicated national network for of food-borne disease may be necessary to obtain the best prevention outcomes. Fifth, statistical models for the attribution of human SA infections from animals (pigs, cattle, and broilers/eggs), and specific foods may be useful to gain a global vision of the agent, reservoirs, and mechanisms of transmission to carry out specific preventive actions[19,32-35].

Conclusions

This study suggests that SA infections mostly affected young children and ST infections were associated with the consumption of pork products.

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