

Nationwide outbreak of *Salmonella* serotype Kedougou associated with infant formula, Spain, 2008

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On 5 August 2008, the National Centre of Microbiology in Madrid, Spain, notified an increase in *Salmonella* Kedougou isolations compared to 2007, with 21 cases including 19 children under one year of age. Active case finding and a matched case-control study were carried out to confirm this increase, identify source, transmission mode and risk factors in order to implement control measures. Cases were defined as any child under one year of age with *S. Kedougou* isolated since 1 January 2008, and were matched for age, sex, medical practitioner and diagnosis week with controls who were selected among patients of the cases' medical practitioners. An *ad hoc* questionnaire was completed for cases and controls and a univariate analysis was conducted to identify risk factors. We found 42 isolates from 11 of the 19 Spanish Regions. Completed questionnaires were available for 39 of 42 patients identified; 31 were children under one year of age and fulfilled the case definition. The median age of the 31 cases was 4.3 months and 13 were male. Main symptoms were diarrhoea ($n=31$) and fever ($n=13$). Ten cases required hospitalisation. All 31 cases had consumed infant formula milk of Brand A which was associated with illness in the univariate analysis (exact matched odds ratio: 74.92; 95% confidence interval: 12.89- ∞). All patient isolates showed indistinguishable pulsed-field gel electrophoresis and antimicrobial susceptibility patterns. Five milk samples from three cases' households were negative for *Salmonella*. Our results suggest that Brand A was the transmission vehicle of *S. Kedougou* in the outbreak that occurred in Spain between January and August 2008. Food safety authorities recalled five batches of Brand A milk on 26 August 2008. No further cases have been detected as of 15 September 2009.

Background

Salmonella Kedougou belongs to serogroup G *Salmonella* and is one of the nearly 2,000 *Salmonella*

serotypes that can cause illness in humans, but it is a rare serotype identified in Spain. The National Centre of Microbiology (NCM) in Madrid isolated a mean of three *S. Kedougou* strains from humans per year between 2002 and 2007 (unpublished data). We only found two outbreaks involving this serotype in the literature: one in Norway in 2006 linked to consumption of Salami [1] and one in the United Kingdom in 1992 linked to cooked meat [2].

On 5 August 2008, the NCM notified an increase in number of *S. Kedougou* isolates during the first half of 2008: 21 isolates from seven Spanish regions, compared to six isolates in 2007 and two in 2006. Nineteen of these 21 isolates were from children under one year of age. The widespread distribution and the cases' age suggested a commercial infant food product as the likely vehicle of transmission in this *Salmonella* outbreak.

On 6 August 2008, the National Centre of Epidemiology (NCE) in collaboration with NCM, regional epidemiologists and microbiologists of the Spanish epidemiological surveillance network began an epidemiological study and sent an alert to the Spanish Food Safety and Nutrition Agency (SFSNA) and to the Ministry of Health. The alert was also sent to the European Food and Waterborne Diseases Network, asking for *S. Kedougou* increases during 2008. Eleven member countries answered but did not report any increase in *S. Kedougou* isolates.

The objectives of our study were to confirm the increase in number of cases and to identify the source of infection, the transmission mode and associated risk factors in order to implement appropriate control measures.

Materials and methods

Epidemiological investigation

An active case finding and a matched case-control study were conducted by the NCE in collaboration with NCM and regional and local epidemiologists to test the hypothesis that consumption of commercial infant food product was associated with the illness.

Active Case Finding

An outbreak case was defined as any person with an isolate of *S. Kedougou* identified during 2008. NCE sent a request to all regions in Spain through the Spanish Epidemiological Surveillance Network, to notify any case from whom *Salmonella* Group G was isolated in 2008.

We collected information on the cases with confirmed *S. Kedougou* infection using a structured questionnaire. They were filled in by regional and local epidemiologists in interviews with the cases or their parents.

We asked for demographic information (age, sex, place of residence), clinical information (date of onset, main symptoms, severity, hospitalisation) microbiological information, human and/or animal contact, food consumed in the 72 hours before the onset of symptoms (including brands and batch numbers of infant food consumed), and information about the way of preparation and disinfection as well as the time from preparation to consumption.

The epidemiological data and food history of the first identified cases (see below) raised the hypothesis that consumption of infant formula could be the cause of infection and we started an analytical study.

Analytical study

A case was defined as any child under one year of age with *S. Kedougou* isolated between 1 January 2008

FIGURE 1

Cases of *Salmonella* Kedougou by region, Spain 2008 (N=42)

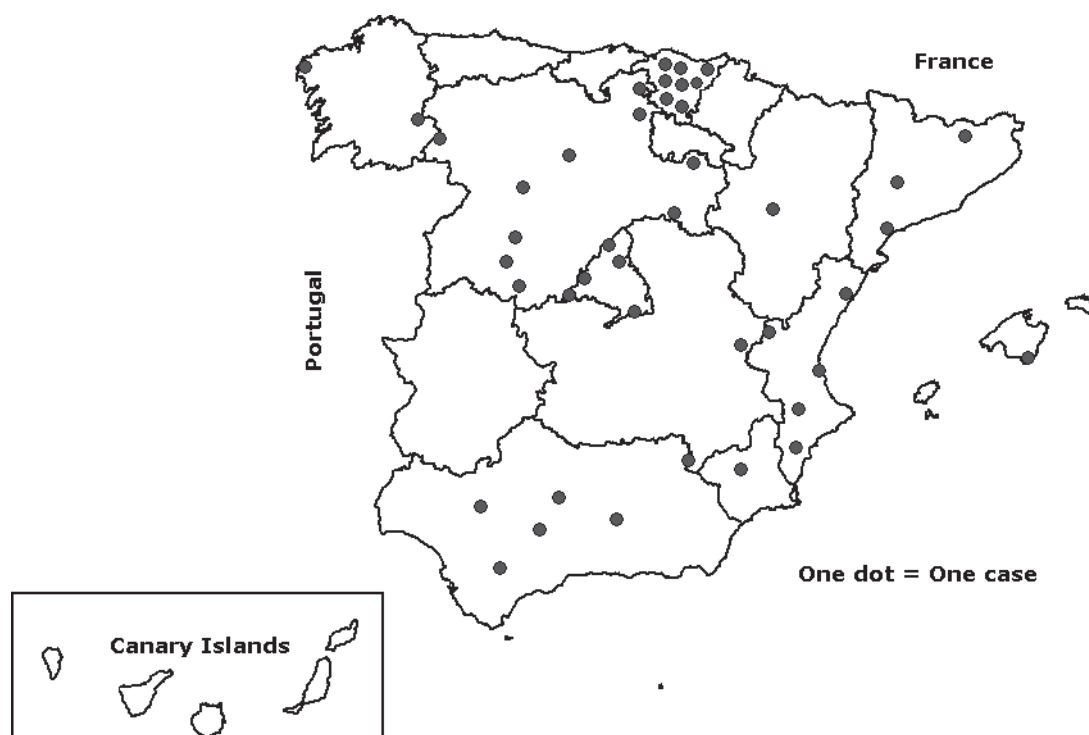
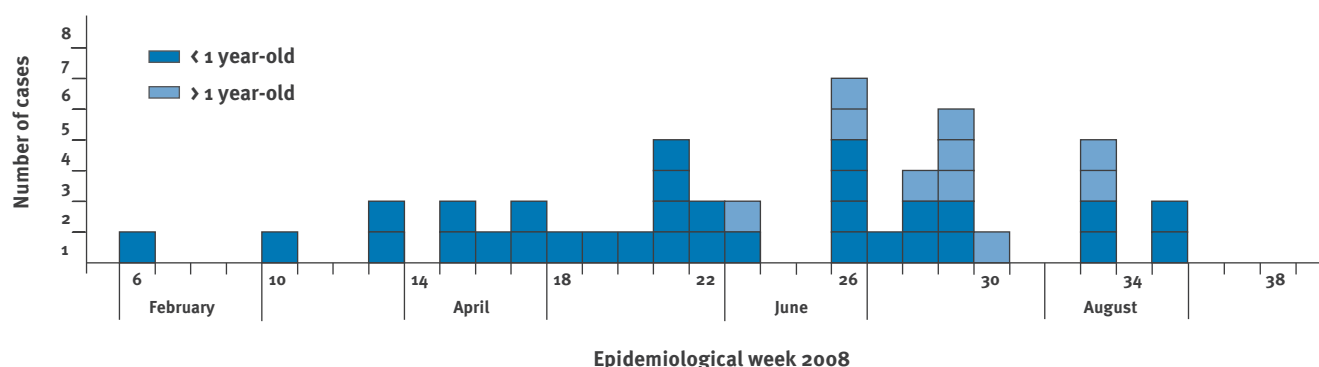


FIGURE 2

Cases of *Salmonella* Kedougou by week of isolation and age, Spain, 2008 (N=42)



and 31 August 2008. For each case, four controls were selected and matched for age ($\pm$ one month), sex, same medical practitioner and week of diagnosis ($\pm$ one week), without gastrointestinal symptoms and non-exclusive breastfeeding. The same questionnaire was applied to cases and controls, asking for information on their food intake during the three days previous to the onset of symptoms of the case.

Statistical analysis

Odds ratios (OR) and their 95% confidence intervals (CI) for the association between risk factors and disease were estimated using exact conditional logistic regression [3]. Maximum likelihood estimates (MLE) were applied when possible, and median unbiased estimates (MUE) when MLE could not be calculated. All analyses were carried out using STATA 10.0 [4-5].

Microbiological Investigation

Strains isolated from cases at regional hospital laboratories were sent to NCM for serotyping, and comparison of pulsed-field electrophoresis (PFGE) profiles and susceptibility patterns. The strains were tested for susceptibility to ampicillin, cefalotin, cefotaxime, amoxicillin/clavulanic acid, chloramphenicol, gentamicin, kanamycin, nalidixic acid, ciprofloxacin, tetracycline and trimethoprim/sulfamethoxazole.

Samples of infant foods (opened or unopened) provided by cases' households were also collected and sent to the laboratory of the SFSNA and to the regional laboratories for *Salmonella* testing.

Results

A total of 42 isolates from 42 patients were identified from January to August 2008. Sixteen patients were

male and 32 were children under one year of age. Ten children under one year of age and a pregnant woman required hospitalisation. Patients were from 11 of the 19 regions in Spain (Figure 1).

The first isolate of *S. Kedougou* was identified on 4 February 2008 and the last one on 29 August 2008 (Figure 2).

Questionnaires for the children were answered by the parents. Completed questionnaires were available for 39 of 42 patients identified. For further analysis, we only considered cases under the age of one year. These were 31 of the 39 respondents, with a median age of 4.3 months, and 13 were male. Main symptoms were diarrhoea ($n=31$), fever ($n=13$) and vomiting ($n=7$). Blood was present in the stools of 20 of the patients. Ten children were hospitalised; none of them had a history of immunosuppression.

Five children had a mixed diet (breast feeding and infant formula) and 26 used infant formula exclusively. All the infants had consumed the same milk, Brand A, in the 72 hours before the onset of symptoms. Table 1 shows products consumed by the patients in the 72 hours before onset of symptoms, as well as other exposures.

The eight patients over one year of age, for whom a completed questionnaire was available, had a median age of 28 years (range: 1-84 years of age). Two patients were parents of cases under one year of age. Three patients had consumed powder infant formula of Brand A in the 72 hours before the onset of symptoms.

We included 22 cases and 70 controls in the matched case-control study (12 cases were matched with four controls, two cases with three controls and eight cases with two controls).

All cases included in this analytical study consumed infant formula of Brand A in the 72 hours before onset of symptoms compared with seven (10%) of the controls. Because all cases consumed an infant formula of Brand A, the maximum likelihood estimation for the OR of association with illness was infinite. The median unbiased estimate for the Mantel-Haenszel OR (OR_{M-H}) and the lower limit of the CI were thus calculated (exact OR_{M-H} : 74.92; 95% CI: 12.89- ∞). Other food products, food preparation, or preservation and disinfection habits were not associated with the disease (Table 2).

Antimicrobial susceptibility tests and PFGE were done on all the strains. All PFGE pattern were indistinguishable (SAL-XBA-KDG-1) and all strains had the same sensitivity profile to all antibiotics tested.

We tested five samples of milk consumed before the occurrence of symptoms and provided by three cases' families. *Salmonella* was not detected in any of them.

TABLE 1

Distribution of exposures during the 72 hours before onset of symptoms in children under one year of age with isolation of *Salmonella* Kedougou, Spain, 2008 (N=31)

Product	N
Breast feeding	5
Milk of Brand A	31
Water	
Tap water	5
Bottled water	24
Baby cereal	11
Baby puree	
Fruit (homemade)	7
Fruit (commercial food)	5
Vegetables and chicken (homemade)	8
Baby bottle disinfection	
Boiling bottles	10
Using steriliser	11
Consumption of formula milk, cereal or puree immediately after preparation	28
Animal contact	9

No further cases' families were able to provide the batch number of the product consumed.

The infant formula Brand A distributed in Spain up to the day of the study had been produced in a local production plant. The company had closed this production plant in March 2008, five months before the outbreak alert. The results of factory quality control tests provided by the producers from raw materials and incriminated end products were negative for *Salmonella*, but positive for *Enterobacteriaceae* in some batches of end product.

On 26 August, the Spanish food safety authorities recalled five batches of infant formula of Brand A. This product was distributed only in Spain. A press release was issued informing people to avoid the use of these batches of milk Brand A and a contact telephone help line was set up to provide information to consumers.

Discussion

Our results suggest that the consumption of an infant formula of Brand A was associated with *S. Kedougou* infection. In our analytical study, 100% of the cases had consumed this milk compared with only 10% of the controls.

Outbreaks associated with infant powder formula are not uncommon because this is not a sterile product. This type of feeding is now usual because of many reasons such as the increased survival of premature babies and newborns with low birth weight, maternal illnesses in which breastfeeding is not recommended, early return of women to work after giving birth, or difficulties in breastfeeding [6].

We are not aware of any outbreak of *S. Kedougou* associated with infant formula. However, other serotypes of *Salmonella* had been associated with infant formula as a vehicle of transmission in many outbreaks in the world, such as in France (*S. Agona*, 2005), Korea (*S. London*, 2000), Spain (*S. Virchow*, 1994), Canada and the United States (*S. Tennessee*, 1993) [7-16]. One of the latest outbreaks occurred in France at the time of our study (*S. Give*, 2008) [17].

Outbreaks associated with commercial products like infant formula tend to have a low epidemic profile (small number of cases spread over long periods of time) because of the low bacterial load usually contained in the product [11]. However, continuous exposure to the factor for several months increases the probability of infection. The current Codex Alimentarius specification for *Salmonella* considers food products as fit for consumption when 60 samples of 25 gr are free from microorganisms [18]. Data provided by the infant food industry and inspection authorities indicate that *Salmonella* is rarely detected in powder infant products; nevertheless the microorganism can survive in powdered formula milk for up to 15 months and the method of detection can fail [19].

The increase in *S. Kedougou* isolations in Spain in 2008 was detected by the NCM because of the low expected frequency of this serotype in our country. This highlights the crucial role of the microbiology laboratories detecting outbreaks involving rare serotypes of microorganisms. Laboratory networks with a role in early detection of alert signals can complement surveillance systems in detecting uncommon microorganisms that otherwise might go unnoticed.

The higher attack rate in children under the age of one year, the identical PFGE pattern, the wide geographical distribution in Spain and the consumption of a particular brand of infant formula by the first cases identified lead to the hypothesis that the milk could be the vehicle of infection. Moreover, almost all cases older than one year could be explained by consumption of Brand A milk or by epidemiological link with younger cases.

In our study, most adult cases were probably secondary cases. Those cases for whom no contact with children under the age of one year could be established had consumed Brand A formula milk. By restricting our study to cases under one year of age we increased the specificity of the case definition because only primary cases were included. In case-control studies recall bias usually differs between cases and controls; parent cases tend to recall better than parent controls. In our study, we minimised this bias by restricting the

TABLE 2

Matched univariate analysis between *Salmonella* Kedougou infection and different exposures, Spain, 2008

Variable	Matched OR	95% CI
Tap water	2.11	0.43–10.22
Fruit baby food	0.8	0.08–7.51
Baby puree (vegetables and chicken)	0.34	0.01–26.07
Infant formula milk Brand A	74.92^a	12.89–∞
Boiled water for baby bottle	5.77	0.95–35.02
Disinfection: water and detergent	0.48	0.12–1.92
Disinfection: boiled water	2.07	0.54–7.85
Animal contact	0.98	0.3–3.12

CI: confidence interval; OR: odds ratio.

^a Median unbiased estimates; maximum likelihood estimation= infinite.

analysis to cases under the age of one year, for whom food consumption patterns are usually constant and thus less prone to recall bias. However, some bias could be present given the delay between the interview and the onset of symptoms (mean: 108 days, range: 9-222 days).

The matched case-control study design chosen [20] included matching for medical practitioner as a way to facilitate the control search and selection. This could have led to overexposure among controls because doctors could tend to recommend the same infant formula to their patients. Nevertheless this assumption was not confirmed by our data, as only few controls consumed the involved milk brand compared with 100% of the cases.

In the case-control questionnaires, up to five varieties of Brand A milk were reported, but the small number of exposed controls did not allow further analysis to identify a specific variety associated with the outbreak. Except in two cases, it was not possible to obtain details on the batch of formula milk consumed by the patients.

The number of cases confirmed at the laboratory (42 cases) might be an underrepresentation of the real number of infections since only a small proportion of people with gastroenteritis seeks medical assistance and provide a sample for laboratory testing. This might be also applicable for the age group at risk (under one year) because even when parents seek medical assistance for their children, gastrointestinal illness might be frequently misdiagnosed as milk/food intolerance as initially happened in two of the cases identified.

This is the first outbreak of *S. Kedougou* associated with the consumption of infant formula in Spain. The results of the investigation involving epidemiological services of all Spanish regions and the NCE support the hypothesis that the Brand A formula milk was the vehicle of the *S. Kedougou* gastroenteritis outbreak, occurring between February and August 2008.

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