RESEARCH ARTICLE **(Open Access)**

Prevalence of *Salmonella spp*. in Imported Powered Infant Formula (PIF)

RENIS MAÇI[1]*, BIZENA BIJO[2], ARDIAN XINXO[1], FATMIRA SHEHU[2], HALIT MEMOÇI[1]

[1]Department of Food Microbiology, Food Safety and Veterinary Institute "Dr. Bilal Golemi", "Aleksandër Moisiu" No. 10, Tirana, Albania
[2]Department of Veterinary Public Health, Faculty of Veterinary Medicine; Kodër-Kamëz, Tirana, Albania

Abstract

Salmonella species are well-known long-standing foodborne human pathogen that demonstrate long-term survival in/on dry or low-water activity (a_w) in foods. Salmonellosis caused by ingestion of contaminated powdered infant formula has been reported nationwide. In recent years, 8 reported outbreaks of *Salmonella* infection in infants have been linked to the consumption of powdered infant formula. Outbreaks of Salmonellosis due to contaminated PIF are likely to be under-reported nationwide even in Albania.

The aim of this study was to investigate the potential existence of *Salmonella spp*. in canned powdered infant formula in Albania. During two years investigation *Salmonella spp* was on the focus and was detected in 1 out of 70 analysed samples (1.43%). The strain of *Salmonella spp.* was biochemically identified by the analytical profile index (API 20 E) system and poly A, H, and Vi antiserum.

Food safety criteria are laid down in EU regulation "EC No. 2073/2005" for *Salmonella spp*. in dried infant formula and dried dietary foods for special medical purposes intended for infants. These criterias are transposed to Albanian Legislation.

A laboratory-based on food-borne disease surveillance systems is needed in terms of strethening control and reducing the risk of exposure.

Keywords: *Salmonella spp*., outbreaks, RASIFF, food microbiology criteria.

1. Introduction

Opinions on the microbiological risks in infant formulae and follow-on formulae issued by The Scientific Panel on Biological Hazards (BIOHAZ Panel, EFSA) came to the conclusions that *Salmonella* and *Enterobacter sakazakii* are the micro-organisms of greatest concern in infant formulae and formulae for special medical purposes [6].

According to Centers for Disease Control and Prevention (CDC 2004) is estimated that the incidence rate of Salmonellosis (from all sources) among infants is 139.4 cases per 100 000. It was reported to be more than eight times greater than the incidence across all ages in the United States of America [5].

In addition during the last decade Rapid Alert for Food and Feed System (RASIFF) have been reported four contaminated cases of infant formula with *Salmonella.* Outbreaks of Salmonellosis due to contaminated PIF are likely to be under-reported.

Over the two last decades, have been published multiple reports which associate bacterial contamination of dried formula with infection in infants fed with these products. *Salmonella* species as members of *Enterobacteriaceae* family have been identified in most of these outbreaks.

The most recently *Salmonella* outbreaks conclude the association with powdered infant formula consumption. These outbreaks are caused by different serotypes such as *S. enterica*, *S. kedougou*, *S. derby*, *S. tennessee*, *S. bredeney*, *S. aaling. S. virchow*, *S. anatum and S. agona* [1,10, 11, 12, 13, 17, 18, 20, 21].

*Corresponding author: Renis Maçi; E-mail: renismaci@hotmail.com
(Accepted for publication on Septembre 25, 2015)
***ISSN**: 2218-2020,* *© Agricultural University of Tirana*

Table 1 History of Salmonellosis outbreaks and their serovars linked with Powdered Infant Formulae (1985-2008)

***Salmonella* serotype**	**No. infants affected**	**Vehicle**	**Location**	**Year**	**Reference**
ealing	48	PIF	UK	1985	Rowe et al., 1987
tennessee	> 3	PIF	USA, Canada	1993	CDC, 1993
virchow	48	PIF	Spain	1994	Usera et al., 1996
anatum	17	PIF	UK, France	1996-7	Threlfall et al., 1998
london	30	PIF	Republic of Korea	2000	Park et al., 2004
agona	141	PIF	Frace	2005	Brouard, C., E. Espie, et al. (2007)
kedougou	21	PIF	Spain	2008	Soler, P., S. Herrera, et al. (2008).
enterica	3	PIF	France	2008	Jourdan, N., S. Le Hello, et al. (2008).

During food processing might occur a significant food safety risk when a certain contamination takes place. The food such as PIF even with low-moisture is in risk during storage. The degree of demage is depended on factors such as storage temperature and product formulation [16].

Poor sanitation practices, not adeguate equipment design and low ingredient control are other factors that contribute in cross contamination of powdered infant formulae.

The heat resistance of *Salmonella* is affected by many factors, such as serotypes , previous growth, storage conditions, physical and chemical food composition, media types . In general *Salmonella* rezistence against heat increases with the moisture reduction[16].

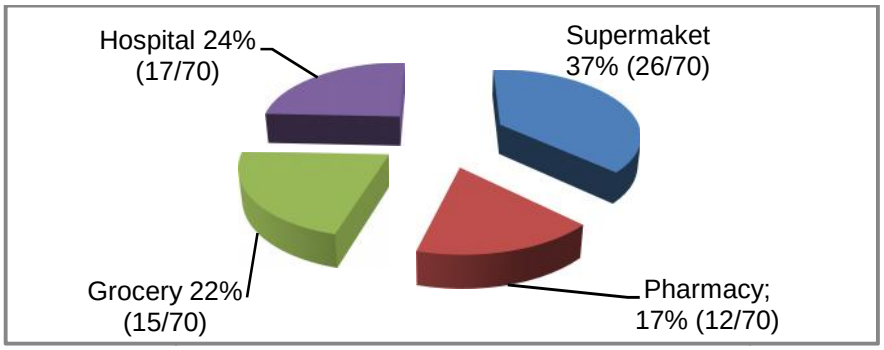

Figure 1. Sample source (n=70).

2. Material and Methods

2.1. Sampling

During the two years study, 70 unopened canned PIF were ramdomly collected at grocery shops (21%), hospital (24%), supermarket (37%) and pharmacy (17%). Every month were transported three samples. The sampling distribution is shown in the figure 1.

In accordance with criteria of PIF producers are analyzed two category of samples. The first category is composed from designated PIF for neonates and children aged one year old (n=40) and the second category aged from 1 to 3 years old (n=30).

Samples were prelieved by food authority officers and by the controllers of Food Business Operators. Samples were transported to Food Safety and Veterinary Insitute (FSVI “Dr. Bilal Golemi”) at the lab of Food Miicrobiology.

Our test results shown a positive sample tested for *Salmonella spp*. Only one sample out of 69 (98,6%) was considered unsatisfactory for *Salmonella spp*. (based on microbiological criteria specified in

Commission Regulation (EC) No. 2073/2005 for foodstuffs [6].

2.2. Sample preparation and incubation

A classical culture method for detecting *Salmonella spp* was performed using Stadard method such as ISO 6579: 2002/Corr1: 2004 [2] that include four steps (pre-enrichment in non-selective liquid medium; selective enrichment in liquid media; plating on selective media; serological and biochemical identification of suspected colonies).

Samples of 25 g weight were transfered on liquid buffered peptone water at 37°C ±1°C for 18 ±2 hours. After being passed on non-selective pre-enrichment stage the samples were inoculated on Rappaport-Vassiliadis with soya (RVs), and on Muller Kauffmann medium with Novobiocine (MKTTn) in the amount of 0,1 mL and 1,0 mL. The following procedure was respectively the incubation on 41,5°C ±1°C/24 ±3 hours for RVs and 37°C ±1°C/24 ±3 hours for MKTTn.

Further we set the samples on two different selective media such as XLD (Xylose Lysine Deoxycholate) Agar and Hectoen agar and incubated on 37°C ±1°C/24 ±3 hours.

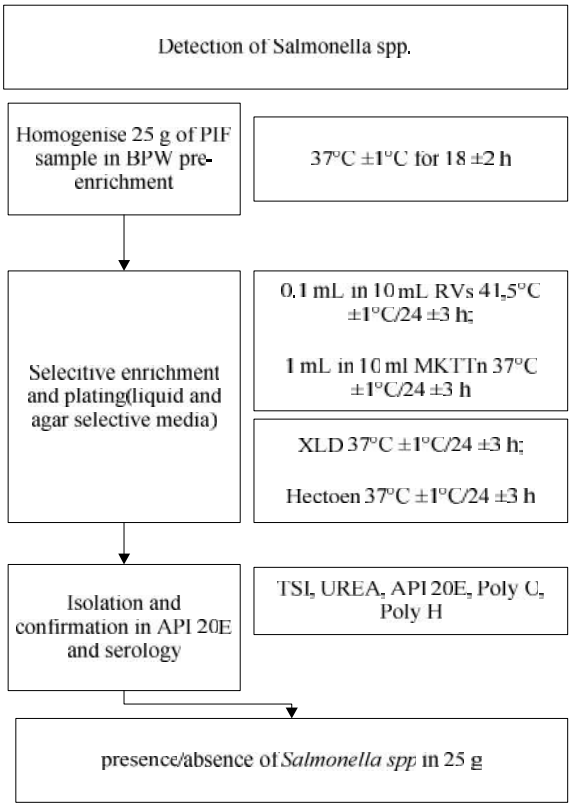

Figure 2. Flow diagram. Detection of *Salmonella spp.* in powdered infant formula(PIF)

At the end of the incubation we identified five suspected colonies which were selected from each plate. These colonies were plated on the media with nutrient agar. After we followed the biochemical and serology examinations. Following carefully the protocol, we used simple biochemical test using Triple-sugar iron agar and urea agar as well as Analytical Profile Index 20E tests (Biomerieux). In accordance with the biochemical features (presence of somatic O, capsular Vi and flagellar H antigens (Becton Dickinson), the selected cultures were classified as *Salmonella* strains.

3. Results and Discussion

The microbiological results of 70 PIF samples are shown in table 2.

The test results identified that 69(98,6%) of canned PIF were found in hygienic condition, respecting the microbiological food criteria. Only one sample (1,4%) taken from supermarket was found unsatisfactory.(based on Albanian Food Regulation).

Table 2. Prevalence of *Salmonella spp.* in PIF during years (2013-2015) (n=70).

Result	*Salmonella* spp. /25g
Absent	69 (98,6%)
Present	1 (1,4%)

In our study we identified that the prevalence of *Salmonella* spp. in powdered Infant formulae was 1,4%. Taking in consideration that in our country *Salmonella* serotyping is not routinely performed, identification of diffused outbreaks could be difficult.

In recent years manufacturers (of powdered infant formulae) have implemented strategies to control *Salmonella* spp. and to reduce risk of exposure.

4. Conclusions

Powdered infant formulas are not sterile products and may contain low levels of *Salmonella* spp.

Routine microbiological investigations are insufficient to detect a low-grade contamination, which may cause serious illness and outbreaks among infants and children. However, in our study we have

identified a higher prevalence of this pathogen compared with surveys in European countries.

Some *Salmonella* serotypes have the potential to cause illness at very low doses, which may be a specific concern for infants, particularly those in the high-susceptibility category (premature, low birthweight, immunocompromised). However, given the thermal resistance of *Salmonella*, reconstituting PIF at a temperature of >70°C or using commercially sterile formula or fortifiers, would provide a high level of protection against *Salmonella* infection from these foodsources.

The safe production of powdered infant formulas is depended on the hygienic level maintance and the control. The control of this food requires a multidimensional approach in which manufacturers, regulators, and caregivers to infants can all play a role.

5. Acknowledgements

The authors wish to thank Institute of Public Health for the support .

6. References

1. Anon. (1993) Centers for Disease Control and Prevention: **Salmonella serotype Tennessee in powdered milk products and infant formula—Canada and the United States**: 1993. MMWR Morb Mortal Wkly Rep 1993; 42:516–7.
2. Anon. (2006) Food and Agricultural Organisation of the United Nations (FAO)/World Health Organisation (WHO): **Enterobacter sakazakii and Salmonella in powdered infant formula: Meeting report. Microbiological Risk Assessment Series 10.**
3. Anon. (2002) ISO 6579:2002 Corr1:2004: **Microbiology of food and animal feeding stuffs -- Horizontal method for the detection of Salmonella spp**. Geneva: *International Organization for Standardization*;
4. Anon. (2003) FDA field survey of powdered formula manufacturing.
5. Anon. (2004) Centers for Disease Control and Prevention: **FoodNet Annual Report 2004.** Available at: http://www.cdc.gov/foodnet/annual/2004/Tables.pdf. Accessed 6 September 2007
6. Anon. (2004) European Food Safety Authority: **Opinion adopted by the BIOHAZ Panel related to the microbiological risks in infant formulae and follow-on formulae**. *EFSA Journal*. 113, 1-35
7. Anon. (2005) Commission Regulation (EC) No 2073/2005 (OJ L338, p1, 22/12/2005): **On microbiological criteria for foodstuffs.**
8. Anon. (2007) World Health Organisation: **Guidelines for the safe preparation and handling of powdered infant formula.**
9. Archer J, Jervis E T, et al. (1998): **Heat resistance of Salmonella weltevreden in low-moisture environments**. *J Food Prot* 61(8): 969-973.
10. Bornemann R, Zerr DM, Heath J, et al.: **An outbreak of Salmonella serotype Saintpaul in a children's hospital***: Infect Control Hosp Epidemiol* 2002; 23:671–6
11. Brouard C, Espie´ E, Weill FX, et al.: **Two consecutive large outbreaks of Salmonella enteric serotype Agona infections in infants linked to the consumption of powdered infant formula**: *Pediatr Infect Dis J* 2007; 26:148–52.
12. Cahill S M, I Wachsmuth, et al. (2008).: **Powdered infant formula as a source of Salmonella infection in infants**: *Clin Infect Dis* 46(2): 268-273.
13. Jourdan N, S. Le Hello, et al. (2008).: **Nationwide outbreak of Salmonella enterica serotype gives infections in infants in France, linked to infant milk formula**: September 2008." *Euro Surveill* 13(39)
14. Koseki S, N Nakamura, et al. (2015).: **Comparison of Desiccation Tolerance among Listeria monocytogenes, Escherichia coli O157:H7, Salmonella enterica, and Cronobacter sakazakii in Powdered Infant Formula**: *J Food Prot* 78(1): 104-110.
15. Park JK, Soek WS, Choi BJ, et al. **Salmonella entericaserovar London infections associated with consumption of infant formula.** Yonsei Med J 2004; 45:43–8. powdered infant formula.
16. Podolak R, E Enache, et al. (2010).: **Sources and risk factors for contamination, survival, persistence, and heat resistance of Salmonella in low-moisture foods:** *J Food Prot* 73(10): 1919-1936

17. Rowe B, Begg NT, Hutchinson DN, et al.: **Salmonella Ealing infections associated with consumption of infant dried milk:** *Lancet* 1987;2:900–3.
18. Soler P, S Herrera, et al. (2008).: **Nationwide outbreak of Salmonella enterica serotype Kedougou infection in infants linked to infant formula milk, Spain 2008**: *Euro Surveill* 13(35).
19. Toyofuku H, K Kubota, et al. (2006).: **Outbreaks of Salmonella in infants associated with powdered infant formula**: *Kokuritsu Iyakuhin Shokuhin Eisei Kenkyusho Hokoku*(124): 74-79.
20. Threlfall EF, Ward L, Hampton M, et al.: **Molecular fingerprinting defines a strain of Salmonella entericaserotype Anatum responsible for an international outbreak associated with formula-dried milk**: *Epidemiol Infect* 1998; 121:289–93.
21. Usera MA, Rodriguez A, Echeita A, Cano R.: **Multiple analysis of a foodborne outbreak caused by infant formula contaminated by a atyp-ical Salmonella Virchow strain**: *Eur J Clin Microbiol Infect Dis* 1998; 17:551–5.
22. Usera M A, A Echeita, et al. (1996). :**Interregional foodborne salmonellosis outbreak due to powdered infant formula contaminated with lactose-fermenting Salmonella Virchow:** *Eur J Epidemiol* 12 (4): 377-381.