2nd Trimester National Microbiological Survey 2006 (06NS2):

Microbiological Safety
of
Dried Infant Formulae and Dried Dietary Foods for Special Medical Purposes Intended for Infants below 6 months of age

Table of Contents

Executive Summary

This study investigated the microbiological safety (*Enterobacter sakazakii* and *Salmonella* spp.) of 'dried infant formulae and dried dietary foods for special medical purposes intended for infants below 6 months of age' on retail sale in the Republic of Ireland.

All single samples (n=719) analysed in this survey meet the limits outlined in Commission Regulation (EC) No. 2073/2005 on Microbiological Criteria for Foodstuffs for *E. sakazakii* (absence in 10g) and *Salmonella* spp. (absence in 25g) in 'Dried infant formula and dried dietary foods for special medical purposes intended for infants below 6 months of age'.

1. Introduction

The World Health Organisation (WHO) recommends that infants are exclusively breastfed for the first 6 months of life and thereafter continue to be breastfed in combination with suitably nutritious complementary foods for up to two years of age or beyond [(1)]. There are however situations where breast milk is not available, where the mother is unable or has made an informed decision not to breastfeed or where breastfeeding is not appropriate [(2)]. In these instances breast milk substitutes are required.

Breast milk substitutes are commercially available in both liquid and powdered form. Although liquid, ready-to-feed infant formula is a commercially sterile product, powdered infant formula (PIF) is not (using current manufacturing technology, it is not possible to produce sterile PIF). PIF may occasionally contain pathogens and has been implicated both epidemiologically and microbiologically as the source and vehicle of infection in infants. *Enterobacter sakazakii* and *Salmonella enterica* are the organisms of most concern. These organisms have been identified by the Food and Agricultural Organisation of the United Nations (FAO) and the World Health Organisation (WHO) as 'Category A Organisms', i.e. organisms present in PIF with a demonstrated causality of illness in infants [(3)].

PIF undergoes a pasteurisation step during its manufacturing process and although *S. enterica* and *E. sakazakii* do not survive the pasteurisation step, recontamination may occur during handling and filling [(4)], i.e. via:

1) the raw materials and in particular the heat sensitive nutrients (e.g. vitamins, minerals etc.) added after pasteurisation
2) the processing environment (i.e. equipment and processing lines) [(3,4,5)].

Although *E. sakazakii* and *Salmonella enterica* cannot grow in PIF, they can survive for a long period of time in the powdered product [(4,5)] and therefore pose a potential risk after rehydration if the product is temperature abused.

E. sakazakii is a Gram negative, non-spore forming bacterium belonging to the *Enterobacteriaceae* family. It has been associated with neonatal meningitis, necrotising enterocolitis (NEC), bacteraemia and necrotising meningoencephalitis [(6)]. It has caused illness in all age groups; however, infants (i.e. children less than 12 months of age) are the group at particular risk. Bowen and Braden (US Centre for Disease Control and Prevention) studied 46 cases of invasive *E. sakazakii* infection to investigate the risk factors relating to ingestion of this pathogen in infants [(7)]**.** Twelve infants had bacteremia, 33 had meningitis and 1 had a urinary tract infection. Infants with meningitis and bacteremia alone fell into 2 distinct groups (Table 1). Those with meningitis had greater gestational age ($p=0.02$), birthweight ($p=0.002$) and developed infection at a younger age ($p<0.001$) than infants with isolated bacteremia. This maybe explained by differences in time of exposure to *E. sakazakii* rather than differences in susceptibility [(5, 7)]. Based on this study, Bowen and Braden suggested that all neonates (infants $\leq$ 28 days) as well as premature infants should be included in the 'high-risk' infant category [(7)]. The FAO/WHO in their risk assessment on *E. sakazakii* and *Salmonella* in powdered infant formula concluded that the high risk group is all infants in the first two months of life [(5)].

Although the number of reported cases of *E. sakazakii* infection in infants is small, this pathogen is of serious concern as the reported mortality rates are high (NEC 10-55% and meningitis 40-80%) [6].

Table 1: Data relating to infants with meningitis and bacteremia alone [7]

Parameter	Symptoms	
	Meningitis	**Bacteremia**
Median gestational ages	37 weeks	27.8 weeks
Median birth weights	2,454g	850g
Median age at infection onset	6 days	35 days

E. sakazakii is ubiquitous in the environment and has been isolated from a diverse range of environments (e.g. processing plants, domestic environments) and foods (e.g.PIF, fermented bread, cheese) [5]. PIF is a demonstrated source of *E. sakazakii* infection in infants. In the CDC study, Bowen and Braden reported that of the 26 infants where feeding patterns were reported 24 were fed on PIF [7]. In New Zealand, a small outbreak of *E. sakazakii* infection linked to PIF was reported in 2004 and resulted in the death of one premature infant. In the same year another outbreak occurred in France, resulting in nine reported cases and two deaths. Worldwide, there have been a number of product recalls of PIF contaminated with *E. sakazakii* since 2004 [5].

Salmonella spp. are Gram negative, facultative anaerobic bacteria belonging to the family *Enterobacteriaceae*. *Salmonella* spp. is an important agent of food borne illness. Symptoms include acute entercolitis with sudden onset of headache, abdominal pain, diarrhoea, nausea, vomiting and fever. Dehydration may occur particularly among vulnerable populations (e.g. infants, the immuno-compromised and the elderly). The condition usually lasts 2-5 days and is generally self limiting [8, 9]. *Salmonella* spp. have caused infection in all age groups; however, infants experience the highest risk of infection. Worldwide between 1985 and 2000, at least 5 outbreaks of salmonellosis involving approximately 150 infants were associated with PIF. In France, 104 infants developed salmonellosis (*Salmonella* Agona) following the consumption of contaminated PIF between January and April 2005 [5]. It is worthy of mention that multiple case control studies have shown that infants who have been breastfed are less likely to have experienced salmonellosis [5].

Strategies to control the microbial contamination of PIF are essential at manufacturing level. Strategies include the application of good manufacturing and good hygienic practices and the implementation of a food safety management system based on the principles of HACCP. The latter is a legal requirement in the EU under Regulation (EC) No 852/2004 on the Hygiene of Foodstuffs [10]. In terms of raw materials, it is essential that ingredients added after heat treatment meet the same microbiological criteria/specifications as the end product. Since the 1st January 2006, food business operators are legally obliged to comply Commission Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs [11]. This Regulation lays down a process hygiene

criterion[ϒ] for *Enterobacteriacaeae* and food safety criteria[⊗] for *Salmonella* spp. and *E. sakazakii* in 'Dried infant formula and dried dietary foods for special medical purposes intended for infants below 6 months of age' (Appendix 1). Food business operators manufacturing these products must also monitor the processing area and equipment for *Enterobacteriaceae*. The presence of *Enterobacteriaceae* in the processing environment is indicative of sporadic low levels of contamination in the final product.

[ϒ] A process hygiene criterion indicates the acceptable functioning of the production process. This criterion is applicable to products at production/manufacturing level. It is not applicable to products placed on the market.

[⊗] A food safety criterion defines the acceptability of a product or a batch of foodstuffs. It is applicable to products placed on the market. When testing against a food safety criterion provides unsatisfactory results, the product or batch of foodstuffs must be withdrawn or recalled in accordance with Article 19 of Regulation 178/2002.

2. Specific Objectives

The aim of this study was to investigate the microbiological safety (*Enterobacter sakazakii* and *Salmonella* spp.) of 'dried infant formulae and dried dietary foods for special medical purposes intended for infants below 6 months of age' on retail sale in the Republic of Ireland.

3. Method

3.1 Sample source

Environmental Health Officers (EHOs) were requested to sample from the following premises:

- Supermarkets
- Grocery shops
- Pharmacies
- Hospitals
- Distributors/importers

3.2 Sample description

Samples included:

- Dried/powdered infant formulae intended for infants below 6 months of age
- Dried/powdered dietary foods for special medical purposes intended for infants below 6 months of age

These samples are hereafter referred to as PIF.

The following were specifically excluded from this survey:

- Dried/powdered infant formulae and dried/powdered dietary foods intended for infants over 6 months of age (e.g. follow-on formulae)
- All liquid formulae (e.g. ready-to-feed formulae)
- All other baby food (e.g. tinned baby food, jarred baby food and cereals)

3.3 Sample collection

Sampling was undertaken by EHOs from the Health Service Executive (HSE) during April, May and June 2006. From every sample source, only one sample of each product was obtained for each producer/supplier (minimum sample weight of 100g). EHOs completed the relevant sections of the accompanying questionnaire (Appendix 2) at the time of sampling.

3.4 Sample analysis

Samples were submitted to the HSE Official Food Microbiology Laboratories (OFMLs) for analysis. Samples were anlaysed for *Salmonella* spp. and *E. sakazakii* using the following methods:

- **ISO 6579:2002** Microbiology of food and animal feeding stuffs -- Horizontal method for the detection of *Salmonella* spp.
- **ISO 22964:2006** Milk and milk products -- Detection of *Enterobacter sakazakii*

3.5 Reporting of Results:

Laboratory results were reported to the FSAI and the relevant EHO as outlined in Table 1:

Table 1: Reporting of results

Microorganism	**Absence or Presence**
***Salmonella* spp.**	Absence or Presence in 25g
Enterobacter sakazakii	Absence or Presence in 10g

3.6 Questionnaire data

Upon receipt of the laboratory results, EHOs completed the questionnaire (Appendix 2) and returned it to the FSAI within 2 months of the survey completion date.

3.7 Statistical analysis

Results were analysed at a 95% confidence interval using the Z-test for two proportions.

4. Results and Discussion

A total of 719 samples submitted from the 10 HSE areas (Appendix 3) were analysed in the 7 OFMLs (Appendix 4). *E. sakazakii* and *Salmonella* spp. were absent in all samples (Table 2, Appendices 5 and 6).

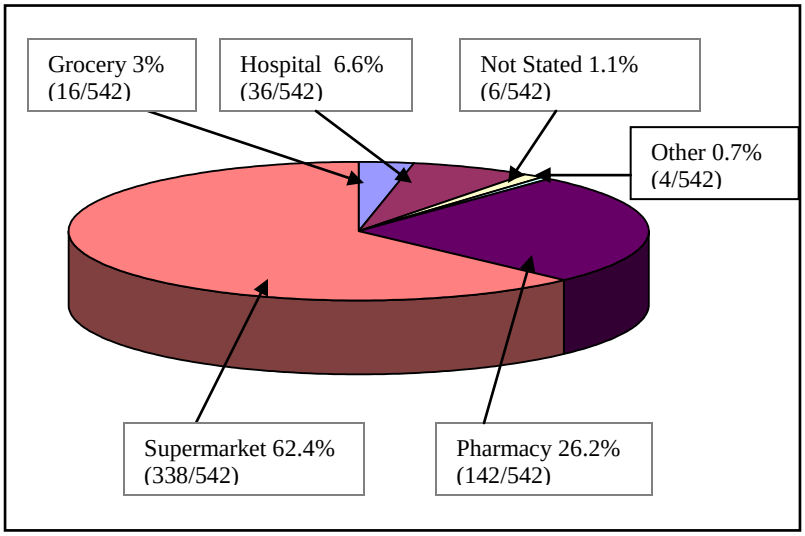
Table 2: Prevalence of *E. sakazakii* and *Salmonella* spp. in PIF (n=719)

Result	No. of samples (%)	
	***E. sakazakii* / 10g**	***Salmonella* spp. /25g**
Absent	719 (100%)	719 (100%)
Present	0 (0%)	0 (0%)

Food safety criteria are laid down in Commission Regulation (EC) No. 2073/2005 on Microbiological Criteria for Foodstuffs [(11)] for *E. sakazakii* and *Salmonella* spp. in 'Dried infant formula and dried dietary foods for special medical purposes intended for infants below 6 months of age' (Appendix 1). Although these criteria are laid down for batch samples (n=30), single samples are permitted when official samples are taken in the context of a monitoring/surveillance programme [(12)]. All single samples (n=719) analysed in this survey meet the limits specified in Commission Regulation (EC) No. 2073/2005 for *E. sakazakii* (absence in 10g) and *Salmonella* spp. (absence in 25g).

Questionnaires were returned for 542 samples, i.e. there was a response rate of 75.4% (542/719). These data show that the samples originated from 8 manufacturers and were sourced predominantly from supermarkets (62.4%, 338/542). Other sample sources included pharmacies, hospitals and grocery shops (Figure 1).

Figure 1: Sample source (n=542)



Other surveys have been undertaken to investigate the prevalence of *E. sakazakii* and *Salmonella* spp. in PIF. Data relating to the prevalence of *E. sakazakii* in PIF are outlined in Table 3. There is a significant difference (p=0.05) between the prevalence of *E. sakazakii* reported in these surveys (2 to 23%) and this Irish survey. However, it should be noted that the results of these surveys were generated using a variety of cultural (using chromogenic or flurogenic substrate in the culture medium) and DNA based techniques. and discrepancies between techniques have been reported [6, 18]. In this Irish survey, samples were anlaysed using the analytical reference methods specified in Commission Regulation (EC) No. 2073/2005 on Microbiological Criteria for Foodstuffs [11].

Table 3: Surveys of *E. sakazakii* in PIF
(Table adapted from the FAO/WHO 2006 risk assessment on *E. sakazakii* and *Salmonella* in PIF [5])

Reference	**Year of publication of survey**	**Number of samples analysed**	**Number of samples with *E. sakazkii* present (%)**
13	1988	141	20 (14.2)
14	1997	120	8 (6.7)
15	2003	22	5 (22.7)
16	2003	101	2 (2)
17	2004	40	5 (12.5)
18	2004	82	2 (2.4)
19	2006	98	12 (12.2)
This study	2007	719	0 (0)

Compared with *E. sakazakii*, *Salmonella* spp. are rarely found in PIF [2].

5. Conclusions

The findings of this study are very encouraging, i.e. *E. sakazakii* and *Salmonella* spp. were absent in all samples (719 single samples). Thus all samples meet the limits for *E. sakazakii* (absence in 10g) and *Salmonella* spp. (absence in 25g) specified in Commission Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs [(11)].

In recent years manufacturers of PIF have implemented strategies to control *E. sakazakii* and *Salmonella* spp. This may explain the absence of these pathogens in this survey compared with earlier surveys.

Control strategies are required not only during manufacture but also during the rehydration, use and handling of PIF as *E. sakazakii* and *Salmonella* spp. (if present) can survive for a long period of time in the dehydrated product. These pathogens can pose a potential risk after rehydration [(4, 5)] (particularly if the rehydrated product is temperature abused). In addition, extrinsic contamination can occur from the preparation environment or from contaminated utensils (e.g. spoons, bottles etc) used for preparing or feeding PIF.

Guidelines on the preparation, use and handling of PIF in care settings and in the home have been published by the FAO/WHO [(2)]. These guidelines are based on a risk assessment which examined the relative risk to infants of different practices for reconstituting PIF and of different feeding practices. In Ireland, information relevant to the development of guidance material for the safe feeding of reconstituted powdered infant formula has been published by the Food Safety Authority of Ireland [(20)].

6. Bibliography

1. World Health Organisation. 2003. Global strategy for infant and young child feeding. http://www.who.int/child-adolescent-health/New_Publications/NUTRITION/gs_iycf.pdf
2. World Health Organisation. 2007. Guidelines for the safe preparation and handling of powdered infant formula http://www.who.int/foodsafety/publications/micro/pif2007/en/index.html
3. Food and Agricultural Organisation of the United Nations (FAO) and the World Health Organisation (WHO). 2004. *Enterobacter sakazakii* and other organisms in powdered infant formula. Microbiological Risk Assessment Series 6. http://www.fao.org/ag/AGN/jemra/enterobacter_en.stm
4. European Food Safety Authority. 2004. Opinion adopted by the BIOHAZ Panel related to the microbiological risks in infant formulae and follow-on formulae. EFSA Journal. 113, 1-35. http://www.efsa.europa.eu/en/science/biohaz/biohaz_opinions/691.html
5. Food and Agricultural Organisation of the United Nations (FAO) / World Health Organisation (WHO). 2006. *Enterobacter sakazakii* and *Salmonella* in powdered infant formula: Meeting report. Microbiological Risk Assessment Series 10. http://www.fao.org/ag/AGN/jemra/enterobacter_en.stm
6. Iversen, C., Forsythe, S. 2003. Risk profile of *Enterobacter sakazakii*, an emergent pathogen associated with infant milk formula. Trends in Food Science & Technol. 14, 443-454.
7. Bowen A.B., Braden C.R. 2006. Invasive *Enterobacter sakazakii* disease in infants. Emerg. Infect. Dis. August Publication http://www.cdc.gov/ncidod/EID/vol12no08/05-1509.htm
8. International Commission on Microbiological Specifications for Foods. 1996. Salmonellae. 217-264. In *Micro-Organisms in Foods. 5. Characteristics of Microbial Pathogens.* Roberts, T.A., Baird Parker, A.C. and Tompkin, R.B. eds. Published by Blackie Academic & Professional, London.
9. Lund, B.M., Baird-parker, T.C. and Gould, G.W. 2000. Salmonella. p1233-1299. In *The Microbiological Safety and Quality of Food. Volume II*. Lund, B.M., Baird-parker, T.C. and Gould, G.W. eds. Published by Aspen Publishers.
10. Regulation (EC) No 852/2004 of the European parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs (L226/3, 25.6.2004) http://www.fsai.ie/legislation/food/eu_docs/Food_hygiene/Reg852_2004.pdf
11. Commission Regulation (EC) No 2073/2005 (OJ L338, p1, 22/12/2005) of 15 November 2005 on microbiological criteria for foodstuffs http://www.fsai.ie/legislation/food/eu_docs/Food_hygiene/Reg852_2004.pdf
12. European Commission. 2006. Guidance document on official controls under Regulation (EC) No 882/2004 concerning microbiological sampling and testing of foodstuffs. http://www.fsai.ie/legislation/food/eu_docs/Food_hygiene/EU_Guidance_882.pdf
13. Muytjens, H.L., Roelofs-Willemse, H., & Jaspar, G.H. 1988. Quality of powdered substitutes for breast milk with regard to the members of the family *Enterobacteriaceae. Journal of Clinical Microbiology*, 26(4): 743-746.
14. Nazarowec-White, M., & Farber, J.M 1997. Incidence, survival, and growth of *Enterobacter sakazakii* in infant formula. *Journal of Food Protection*, 60: 226-230.
15. FDA. 2003. FDA field survey of powdered formula manufacturing. Available at http://www.fda.gov/ohrms/ dockets/ac/03/slides/3939s1_Zink.ppt.
16. Heuvelink, A.E., Zwartkruis-Nahuis, J.T.M., van der A, H., Wit, B., van Oosterom, R., & de Boer, E. 2003. Handhavingsactie *Enterobacter sakazakii* in zuigelingenvoeding. Projectnummer: OT 0210 8 p. Cited by FAO/WHO 2006 (Reference no. 5).

17. Kress, C., Estuningsih, E., Hassan, A.A., Akineden, Ö., Schneider, E., Becker, H., & Uslber, E. 2004. Bedeutung und Nachweis von *Enterobacter sakazakii* in Säuglingsnahrung. 45. Arbeitstagung des Arbeitsgebietes "Lebensmittelhygiene" der Deutschen Veterinär-medizinischen Gesellschaft vom 28 September bis 1 Oktober in Garmisch-Partenkirchen, Tagungsbericht. Cited by FAO/WHO 2006 (Reference no. 5).
18. Iversen, C., & Forsythe, S. 2004. Isolation of *Enterobacter sakazakii* and other Enterobacteriaceae from powdered infant formula milk and related products. *Food Microbiology*, 21: 771-777.
19. Santos, R. 2006. Determination of *Enterobacter sakazakii* in powdered infant formula, reconstituted and utensils used in baby's bottle preparation. Poster P1-38. IAFP (International Association for Food Protection), Calgary, Canada, 13-16 Aug. 2006. Cited by FAO/WHO 2006 (Reference no. 5).
20. Food Safety Authority of Ireland. 2007. Guidance Note No. 22. Information relevant to the development of guidance material for the safe feeding of reconstituted powdered infant formula. http://www.fsai.ie/publications/guidance_notes/gn22.pdf

7. APPENDICES

APENDIX 1

Process hygiene and food safety criteria laid down in *Commission Regulation (EC) No 2073/2005 on Microbiological Criteria for Foodstuffs* [(11)] for *'dried infant formula and dried dietary foods for special medical purposes intended for infants below 6 months of age'*

Table 1a: Process hygiene criterion for *Enterobacteriaceae* in 'dried infant formula and dried dietary foods for special medical purposes intended for infants below 6 months of age'

Microorganism	Sampling plan*		Limit (cfu/g)*		Analytical Reference Method	Stage where the criterion applies
	n	c	m	M		
Enterobacteriaceae	10	0	Absence in 10g		ISO 21528-1	End of the manufacturing process

If *Enterobacteriaceae* are detected the batch has to be tested for *E. sakazakii* and *Salmonella* spp. (see Table 1b).

Table 1b: Food safety criteria for *E. sakazakii* and *Salmonella* spp. in 'dried infant formula and dried dietary foods for special medical purposes intended for infants below 6 months of age'

Microorganism	Sampling plan*		Limit (cfu/g)*		Analytical Reference Method	Stage where the criterion applies
	n	c	m	M		
E. sakazakii	30	0	Absence in 10g		ISO/DTS 22964	Products placed on the market during their shelf life
Salmonella spp.	30	0	Absence in 25g		EN/ISO 6579	Products placed on the market during their shelf life

* n=number of units comprising the sample, c = number of sample units giving values over m or between m and M.

The test results demonstrate the microbiological quality of the process/batch tested:

- satisfactory, if the bacterium is absent in all samples
- unsatisfactory, if the bacterium is detected in any of the samples

NOTE:

For a full interpretation of these criteria please consult Commission Regulation (EC) No 2073/2005 [(11)] and the EU Guidance document on official controls [(12)].

APPENDIX 2

Questionnaire: Microbiological Safety of Dried Infant Formulae and Dried Dietary Foods for Special Medical Purposes Intended for Infants below 6 months of age

General Information:

* EHO Name: _____
* EHO Sample Reference Number *(i.e. EHO's own personal reference number for the sample)* _____
* Laboratory Reference Number *(upon receipt of lab report)* _____

Premises Information (See section 4 of Protocol):

Supermarket	☐	
Grocery shop	☐	
Pharmacy	☐	
Hospital	☐	
Distributor/Importer	☐	Plant No.: _____
Other	☐	Please specify: _____

Sample Information (See section 3 of protocol) :

Manufacturer name & address: _____
_____

Product name: _____
Batch Number: _____
Best before date: _____

Microbiological results (see section 8 of protocol):

	Please tick boxes as appropriate
Salmonella spp.	Absent in 25g ☐ or present in 25g ☐
E. sakazakii	Absent in 10g ☐ or present in 10g ☐

Follow-up action (see section 9 of protocol):

(Follow-up action is only required when Salmonella spp. *and/or E. sakazakii has been detected; please tick as many boxes as necessary)*

Product recall	☐
Product withdrawal	☐
Repeat sample	☐ Lab. ref. no. of repeat sample :_____
Other action	☐ Details: _____
	_____

APPENDIX 3

No. of samples submitted from each Health Service Executive (HSE) Region & Area

HSE Region	HSE Area	Number of samples submitted
HSEDMLR	East Coast Area	24
	Midlands Area	57
	South Western Area	54
HSEDNER	North Eastern Area	60
	Northern Area	31
HSESR	South Eastern Area	195
	Southern Area	103
HSEWR	Mid-Western Area	80
	North Western Area	57
	Western Area	58
Total		**719**

APPENDIX 4

No. of samples analysed in each Official Food Microbiology Laboratory (OFML)

OFML	No. of samples analysed in each OFML
Cherry Orchard	134
Cork	103
Galway	58
Limerick	80
Sligo	57
SPD	92
Waterford	195
Total	**719**

APPENDIX 5
Enterobacter sakazakii results by HSE Area

HSE Area	Presence/absence of *E. sakazakii* in 10g sample		
	Present in 10g	Absent in 10g	Total
East Coast Area	0	24	24
Midlands Area	0	57	57
Mid-Western Area	0	80	80
North Eastern Area	0	60	60
North Western Area	0	57	57
Northern Area	0	31	31
South Eastern Area	0	195	195
South Western Area	0	54	54
Southern Area	0	103	103
Western Area	0	58	58
Total	**0 (0%)**	**719**	**719**

APPENDIX 6
Salmonella spp. results by HSE Area

HSE Area	Presence/absence of *Salmonella* spp. in 25g sample		
	Present in 25g	Absent in 25g	Total
East Coast Area	0	24	24
Midlands Area	0	57	57
Mid-Western Area	0	80	80
North Eastern Area	0	60	60
North Western Area	0	57	57
Northern Area	0	31	31
South Eastern Area	0	195	195
South Western Area	0	54	54
Southern Area	0	103	103
Western Area	0	58	58
Total	**0 (0%)**	**719**	**719**