LACORS/Health Protection Agency PILOT Study: Assessment of the microbiological safety of ready-to-eat shelled nuts from retail premises with a focus on *Salmonella* spp.

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On behalf of the Local Authorities Co-ordinators of Regulatory Services and the Health Protection Agency

Summary

There is little published information on the prevalence of *Salmonella* spp. in ready-to-eat nuts. A pilot study in early 2008 of ready-to-eat shelled nuts on retail sale in England was undertaken to assess the microbiological safety of this product. A total of 727 nut samples of different varieties were examined. Overall *Salmonella* spp. and *Escherichia coli* were detected from 0.2% and 0.4% of ready-to-eat shelled nuts, respectively. Of the nut varieties examined, *S.* Havana was detected from one sample (4%) of pistachio nuts indicating a risk to health. The UK Food Standards Agency was immediately informed and full investigations undertaken. Further examination established the contamination to be associated with the pistachio kernels and not the part opened shells. *Salmonella* spp. was not detected in other varieties tested (almonds, Brazils, cashews, hazelnuts, macadamia, peanuts, pecans, pine nuts, walnuts). *E. coli* was found at low levels (range of 3.6 – 4/g) in walnuts (1.4%), almonds (1.2%), and Brazils (0.5%). The presence of *Salmonella* spp. is unacceptable in ready-to-eat shelled nuts. Prevention of microbial contamination in these products lies in the application of good hygiene practices during growing, harvesting and processing from farm to fork, and effective decontamination. The information from the pilot study will be used to develop the scope of the national LACORS/HPA study planned to start in October 2008.

Introduction

In late 2007, a confectionery firm closed down four production lines after *Salmonella* Schwarzengrund were found in some products (part coated Brazils, final product). None of the affected batches were supplied on to the market. The Company's statement indicated that the contamination was most likely to have come from a batch of Brazil nuts at its plant (Anon, 2007). However, the actual source of contamination was not categorically identified. The pulsed field gel electrophoresis (PFGE) profile of the *S.* Schwarzengrund isolated from the above incident was indistinguishable from that of *S.* Schwarzengrund that caused an outbreak in England, Wales and Scotland from November 2006 to February 2007. However, the outbreak investigation could not ascribe a particular food vehicle to cases of infection (HPA, 2007a).

Following the findings of *Salmonella* spp. in 1% of ready-to-eat dried seeds in the recent LACORS/HPA study (a similar product to ready-to-eat shelled nuts), together with concerns following the above incident, a planned and structured study of ready-to-eat shelled nuts focusing on *Salmonella* spp. is included as one of the food studies within the 2008/9 LACORS/HPA food sampling programme. The start date of the national study is planned as October 2008. To facilitate the development of the scope of the national study, a pilot study took place during January and February 2008. Importantly, the pilot study also allowed an earlier assessment of the microbiological safety of ready-to-eat shelled nuts, focusing on *Salmonella* spp. and *Escherichia coli*.

Materials and Methods

Sample Collection

A total of 727 ready-to-eat shelled nut samples collected as part of the pilot study from retail premises were examined by 18 Official Control Laboratories (HPA & HPA Collaborating) in England from 21 January to 29 February 2008. Nuts coated with chocolate, yoghurt or other coatings, flavoured with seasonings (spices, salt, etc.), or those cooked were specifically excluded from the study. Registered retail premises lists held by Local Authority (LA) Environmental Health Departments (ENDs) were used to derive an approach to sampling. Retail premises were selected at random from LAs' database of food businesses via a random number generator or every 10th entry, and if suitable samples were collected. Samples (≥50g) were collected and transported to laboratories by staff from 103 EHDs, involving 39 Local Authority Food Liaison Groups, in accordance with the Food Standards Agency (FSA) Food Law Code of Practice (FSA, 2006) and the Local Authorities Co-ordinators of Regulatory Services (LACORS) guidance on microbiological food sampling (LACORS, 2006). Information on samples and premises was obtained by observation and

enquiry and recorded on a standard proforma. This included information on the type of nuts, packaging, and country of origin.

Sample Examination

Salmonella spp. and *E. coli* were enumerated or presence sought in accordance with HPA Standard Microbiological Methods (HPA, 2005; 2007b). Isolates of *Salmonella* were sent to the Laboratory of Enteric Pathogens (LEP) at the HPA Centre for Infections, for further characterization and antimicrobial susceptibility testing (Frost, 1994; Ward et al., 1997). Microbiological results were compared to Guidelines for the microbiological quality of some ready-to-eat foods sampled at the point of sale (Gilbert et al., 2000) and requirements within Regulation (EC) No. 178/2002 (General Food Law Regulation) (EC, 2002). Ready-to-eat foods contaminated with *Salmonella* spp. are unsafe; they are considered to be injurious to health and/or unfit for human consumption as they contravene Article 14 of Regulation (EC) No.178/2002 (EC, 2002).

Results

Prevalence of *Salmonella* spp. and *Escherichia coli*

Salmonella spp. were detected from 0.2% (1) of 727 ready-to-eat nut samples, which was of unacceptable microbiological quality. All samples were of satisfactory quality for levels of *E. coli.* *Escherichia coli* was only present in three samples (0.4%) of ready-to-eat nuts and at low levels (ranging from 3.6 – 4 /g).

Details of the ready-to-eat nuts and the *Salmonella* serotype and *E. coli* levels are provided in Table 1.

- *S*. Havana was detected in one sample (4%) of ready-to-eat pistachio nuts. The UK Food Standards Agency was immediately informed, the affected batch was recalled and full investigations undertaken.
- *E. coli* was found in low levels in walnuts (1.4%), almonds (1.2%), and Brazils (0.5%).

Further laboratory investigation of the contaminated pistachio nuts by the HPA London Food, Water & Environmental Microbiology Services Laboratory revealed that *S.* Havana was present on the kernals but not detected on the part opened shells. *Salmonella* Havana infection is rare in England and Wales, with 57 cases reported from 2004 to date. Although there was one case of human *S.* Havana infection during January and February 2008, it is not known whether this infection was linked to consumption of nuts.

Table 1. *Salmonella* and *E. coli* isolated from ready-to-eat shelled nuts from retail premises

Type of shelled nut	Packaging	Country of origin	Best before date	Date sampled	*Salmonella* serotype	*E. coli*/g
Pistachio	Pre-packed	Produce of >1 country	Jan 2009	25/02/2008	*S*. Havana [13,23: f.g:-]	<3
Almonds	Pre-packed	Produce of >1 country	01/09/2008	30/01/2008	Not detected	4
Brazils	Pre-packed	Bolivia	Not known	28/02/2008	Not detected	3.6
Walnuts	Pre-packed	India	01/07/2008	21/02/2008	Not detected	4

Details of ready-to-eat shelled nuts examined

The varieties of ready-to-eat shelled nuts sampled in the pilot study are presented in Table 2. A greater proportion of single types were sampled (91.3%), of which most were Brazils (32.8%), cashews (19.6%), almonds (12.5%), and walnuts (11.1%). Most of the shelled nuts sampled were whole nuts (81.4%), halved/broken nuts (18.5%), or a mix of both whole or halved nuts (0.1%).

Table 2. Ready-to-eat shelled nut varieties collected from retail premises

Variety of nut	No. samples n=727	(%)
Single Type	**664**	**(91.3)**
Almonds	83	(12.5)
Brazils	218	(32.8)
Cashews	130	(19.6)
Hazels	38	(5.7)
Macadamia	14	(2.1)
Peanuts	26	(3.9)
Pecans	25	(3.8)
Pine Nuts	29	(4.4)
Pistachios	25	(3.8)
Walnuts	74	(11.1)
Other (Tiger nuts)	2	(0.3)
Mixed Types	**63**	**(8.6)**
3	7	(11.1)
4	35	(55.6)
5	12	(19.0)
>5	9	(14.3)

The country of origin of ready-to-eat shelled nuts sampled in the pilot study is presented in Table 3. Ready-to-eat shelled nuts sampled were produced in 15 countries with most produced in Bolivia (10.7%).

The packaging format of ready-to-eat shelled nuts sampled in the pilot study is presented in Figure 1; most were pre-packed samples (94.8%). Of the ready-to-eat shelled nuts sampled 11.1% were labelled as organic and 88.9% were not. The premises types visited in the pilot study to collect samples of ready-to-eat shelled nuts are presented in Figure 2. Most shelled nuts were sampled from supermarkets (40.6%) and health food shops (33.0%).

Table 3 Country of origin of ready-to-eat shelled nuts

Country of origin	No. samples n=727	(%)
Bolivia	78	(10.7)
Brazil	29	(4.0)
China	32	(4.4)
Germany	1	(0.1)
India	37	(5.1)
Iran	4	(0.6)
Italy	8	(1.1)
Netherlands	1	(0.1)
Peru	1	(0.1)
South Africa	10	(1.4)
South America (i.e. Brazil & Bolivia; Amazonian rain forest)	13	(1.8)
Spain	8	(1.1)
Turkey	18	(2.5)
USA	53	(7.3)
Vietnam	25	(3.4)
Not known (No details available)	327	(45.0)
Other (Produce of >1 country)	82	(11.3)

Fig. 1 Packaging details of ready-to-eat shelled nuts

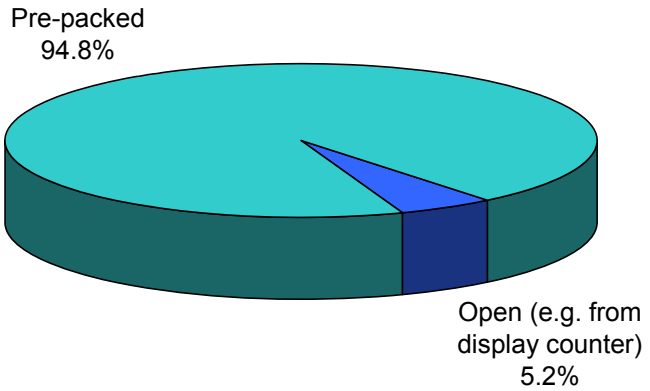
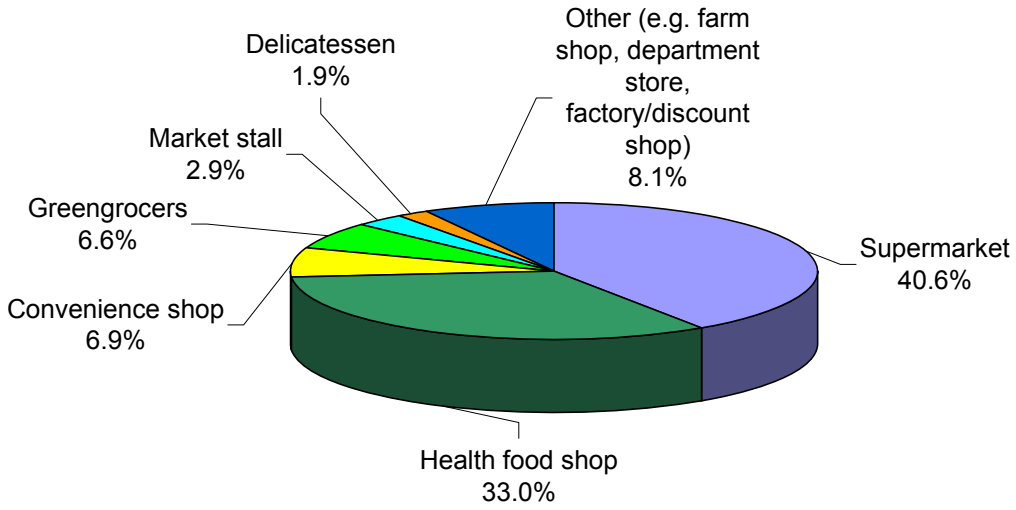


Fig. 2 Premises types visited to collect ready-to-eat shelled nuts



Discussion

Tree nut kernals, such as peanuts, almonds, cashews, hazelnuts, Brazils, have traditionally been considered bacteriologically safe food products due to their low water activity (generally <0.7). The principal microbiological focus has been on managing mycotoxins produced by fungi such as *Aspergillus flavus* and *Aspergillus parasiticus* (Wareing et al., 2000). *Salmonella* spp. has been isolated from peanut, almond, cashew, and Brazil nut kernals (Eglezos et al., 2008; Freire and Offord, 2002; Danyluk et al., 2007; Kirk et al., 2004) and although cannot multiply on nuts, can survive on and in these products for extended periods (greater than 1 year). Recent outbreaks of salmonellosis associated with the consumption of peanuts, peanut products, and almonds have raised awareness of nuts as a potential vehicle for foodborne illness (CDC 2004; Isaacs et al., 2005; Kirk et al., 2004; Ledet Muller et al., 2007).

Salmonella spp. (*S.* Havana) was detected from one sample (4%) of pistachio nuts and further examination established the contamination to be associated with the kernels and not the part opened shells. *Salmonella* spp. was not detected in other varieties tested (almonds, cashews, hazels, macadamia, peanuts, pecans, pine nuts, walnuts). There was also no *Salmonella* spp. detected from Brazil nuts despite the increased scrutiny (a third of nuts sampled were Brazil nuts). This level of scrutiny was deemed necessary because of the high-profile UK *S.* Schwarzengrund chocolate Brazil nuts incident in 2007 (Anon, 2007) and the limited published data on the prevalence of *Salmonella* in this variety of nut. Additionally, as part of a North East regional study on Christmas dried fruits and nuts carried out from mid

December 2007 to early January 2008 23 ready-to-eat nuts (10 of which were Brazils) were examined, none of which contained *Salmonella* spp. (Ian Richardson, HPA Newcastle Environmental Laboratory, pers. comm.).

Escherichia coli is an indicator of faecal contamination. Overall, the *E. coli* prevalence in ready-to-eat nuts was also found to be low (0.4%). *E. coli* levels did not correlate with the presence or absence of *Salmonella* spp. on nuts but there were too few samples in the pilot study with *Salmonella* spp. or *E. coli* present to draw any conclusions on this finding. A US study on raw almonds has however shown that *E. coli* levels did not correlate with the presence of *Salmonella* spp. and therefore would not be a useful indicator organism for this purpose (Danyluk et al., 2007).

Nuts are usually produced in large orchards or plantations, with the exception of Brazil nuts. Brazil nuts are still mainly collected from wild trees in South America. Commercial harvesting of tree nuts is either by hand, or by using mechanical shakers to remove the nuts from the tree, after which they are caught below (Wareing et al., 2000). The prevention of microbial contamination in nuts lies in the application of good hygiene practices during growing, harvesting and processing from farm to fork, and effective decontamination. Codex has produced a Code of Hygienic Practice for tree nut suppliers to help minimize microbial food safety hazards (Codex Alimentarius Commission, 1972). The EC Regulation on the hygiene of foodstuffs (EC, 2004) advocates the principles of hazard analysis and critical control points (HACCP) and the establishment of in-process controls to ensure product integrity, rather than reliance on end-product testing for compliance with specifications. In the tree nut industry the strengthening of HACCP systems that encompass all stages of production, processing and distribution will serve to further enhance the microbial safety of these products.

Acknowledgements

The authors would like to thank all the staff in the Environmental Health Departments throughout England who collected samples for this study, and all the staff in both HPA and HPA collaborating laboratories at Ashford, Birmingham, Bristol, Chelmsford, Chester, Haywards Heath, Leeds, Leicester, Lincoln, London, Newcastle, Norwich, Plymouth, Preston, Sheffield, Shrewsbury, Stoke and WEMS who performed the microbiological examinations. Thanks are extended to LEP, HPA Centre for Infections for typing isolates, and to the HPA Regional Food, Water and Environmental Co-coordinators Forum for advice on the sampling protocols.

References

Anon, 2007. Nut contamination found at plant. BBC News Online, 30 October 2007. Available at: http://news.bbc.co.uk/1/hi/england/leicestershire/7069364.stm. (accessed 16 April 2008).

Centers for Disease Control and Prevention (CDC), 2004. Outbreak of *Salmonella* serotype Enteritidis infections associated with raw almonds – United States and Canada, 2003-2004. MMWR 53, 484-487.

Codex Alimentarius Commission, 1972. Recommended international code of hygienic practice for tree nuts. CAC/RCP 6-1972. Available at: www.codexalimentarius.net/download/standards/266/CXP_006e.pdf (accessed 17 April 2008).

Danyluk, M.D., Jones, T.M., Abd, S.J., Schlitt-Dittrich, F., Jacobs, M., Harris, L.J., 2007. Prevalence and amounts of *Salmonella* found on raw California almonds. J. Food Prot. 70, 820-827.

Eglezos, S., Huang, B., Stuttard, E., 2008. A survey of the bacteriological quality of preroasted peanut, almond, cashew, hazlenut, and Brazil nut kernels received into three Australian nut-processing facilities over a period of 3 years. J. Food Prot., 71, 402-404.

European Commission (EC), 2002. Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Off. J. Europ. Communities L31, 1-24.

European Commission (EC), 2004. Regulation (EC) No. 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. Off. J. Europ. Union L139, 1–54.

Food Standards Agency (FSA), 2006. Food Law Code of Practice. FSA, London. Available at: http://www.food.gov.uk/news/newsarchive/2006/mar/copengland. (accessed 16 April 2008).

Frost J., 1994. Testing for resistance to antibacterial drugs. In: Chart, H. (Ed.) Methods in Practical Laboratory Bacteriology. New York, CRC Press, pp 73-82.

Gilbert, R.J., de Louvois, J., Donovan, T., Little, C., Nye, K., Riberio, C.D., Richards, J., Roberts, D., Bolton, F.J., 2000. Guidelines for the microbiological quality of some ready-to-eat foods sampled at the point of sale. Comm. Dis. Pub. Health 3, 163-167.

Health Protection Agency (HPA), 2007a. An outbreak of *Salmonella* Schwarzengrund in England and Wales November 2006 to February 2007. Health Protection Report 1, 16 March 2007. Available at : http://www.hpa.org.uk/hpr/archives/2007/news2007/news1107.htm#salm. (accessed 16 April 2008).

Health Protection Agency (HPA), 2007b. Detection of *Salmonella* species. National Standard Method F 13 Issue 3. Available at: http://www.hpa-standardmethods.org.uk/documents/food/pdf/F13.pdf. (accessed 12 April 2008).

Health Protection Agency (HPA), 2005. Enumeration of β-glucuronidase positive *Escherichia coli* - Most Probable Number Method. National Standard Method F22 Issue 1. Available at: http://www.hpa-standardmethods.org.uk/documents/food/pdf/F22.pdf. (accessed 16 April 2008).

Isaacs, S., Aramini, J., Ciebin, B., Farrar, J.A., Ahmed, R., Middleton, D., Chandran, A.U., Harris, L.J., Howes, M., Chan, E., Pichette, A.S., Campbell, K., Gupta, A., Lior, L.Y., Pearce, M., Clark, C., Rodgers, F., Jamieson, F., Brophy, I., Ellis, A., *Salmonella* Enteritidis PT 30 Outbreak Investigation Group, 2005. An international outbreak of salmonellosis associated with raw almonds contaminated with a rare phage type of *Salmonella* enteritidis. J. Food Prot. 68, 191-198.

Kirk, D.M., Little, C.L., Lem M., Fyfe, M., Tan, A., Threlfall, J., Paccagnella, A., Lightfoot, D., Genobile, D., Li, H., McIntyre, L., Crawford, C., Ward, L., Brown, D.J., Surman, S.,

Fisher, I.S.T., 2004. An outbreak due to peanuts in their shell caused by *Salmonella enterica* serotypes Stanley and Newport—sharing molecular information to solve international outbreaks. Epidemiol. Infect. 132, 571-577.

Ledet Muller, L., Hjertqvist, M., Payne, L., Pettersson, H., Olsson, A., Plym Forshell, L., Andersson, Y., 2007. Cluster of *Salmonella* Enteritidis in Sweden 2005-2006 – suspect source: almonds. Euro Surveill 12, E9-10.

Local Authorities Co-ordinators of Regulatory Services (LACORS), 2006. LACORS Guidance Food Sampling for Microbiological Examination, Issue 2. Available at: http://www.lacors.com. (accessed 16 April 2008).

Ward L.R., de Sa J.D., Rowe B., 1987. A phage-typing scheme for *Salmonella* Enteritidis. Epidemiol. Infect. 99, 291-294.

Wareing, P.W., Nicolaides, L., Twiddy, D.R., 2000. Nuts and nut products. In: Lund, B.M., Baird-Parker, T.c., Gould, G.W. (Eds) The microbiological safety and quality of food, vol 1. New York, Springer, pp 919-940.

Annex 1: Participating Laboratories and Local Authority Food Liaison Groups and number of samples

Table I. Participating HPA and HPA Collaborating Laboratories and number of samples

HPA Region	HPA/HPA Collaborating Laboratory	No. samples
East	Chelmsford	40
	Norwich	38
East Midlands	Leicester	50
	Lincoln	27
London	London FWEM[1]	88
South East	Ashford	55
	Haywards Heath	60
	WEMS[2]	35
North East and	Leeds	70
Yorkshire and the	Newcastle	64
Humber	Sheffield	41
North West	Chester	10
	Preston	19
South West	Bristol	28
	Plymouth	15
West Midlands	Birmingham	30
	Shrewsbury	28
	Stoke	29
Total		**727**

1, London Food, Water & Environmental Microbiology Services Laboratory
2, Wessex Environmental Microbiology Services

Table II: Participating Food Safety Liaison Groups and number of samples

Local Authority Food Liaison Group	No. samples
Berkshire	13
Buckinghamshire	10
Cambridgeshire	12
Cheshire	4
Derbyshire	23
Devon	15
Durham	12
East Sussex	11
Essex	28
LFCG[1] Greater London NE Sector	5
LFCG Greater London NW Sector	24
LFCG Greater London SE Sector	24
LFCG Greater London SW Sector	9
Greater Manchester	7
Hampshire & Isle of Wight	7
Hereford & Worcester	15
Hertfordshire & Bedfordshire	22
Humberside	13
Kent	55
Lancashire	6
Leicestershire	50
Lincolnshire	18
North Wales	6
North Yorkshire	31
Norfolk	20
Nottinghamshire	9
Northumberland	5
Oxfordshire	15
Shropshire	10
South West Yorkshire	47
Staffordshire	34
Suffolk	18
Surrey	45
Tees Valley	19
Tyne & Wear	25
West Midlands	28
West of England	21
West Sussex	4
Wiltshire	7
Total	**727**

1, London Food Co-ordinating Group