CONFIDENTIAL

FINAL REPORT

1st November 2010 to 31 October 2011

A co-ordinated monitoring programme on the prevalence of *Listeria monocytogenes* in certain ready-to-eat foods to be carried out in the Member States

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UNITED KINGDOM

Food Standards Agency

Aviation House

125 Kingsway

London WC2B 6NH

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A co-ordinated monitoring programme on the prevalence of *Listeria monocytogenes* in certain ready-to-eat foods to be carried out in the Member States

Project Summary

The European Food Safety Authority and its Task Force on Zoonoses Data Collection were requested by the European Commission to produce a technical specification on a co-ordinated monitoring programme (a survey scheme) for *Listeria monocytogenes* in ready-to-eat (RTE) foods. This survey will allow the comparison of *L. monocytogenes* contamination in RTE foods in all EU Member States and the verification of the application of the Community food safety criteria *for L. monocytogenes*. The study focussed on RTE food in which the highest rates of *L. monocytogenes* contamination have been observed in the European Union (EU): soft and semi-soft cheeses, hot and cold smoked and gravad fish, and heat-treated meat products that are handled after heat treatment.

As part of this co-ordinated EU project, 1,600 food samples were collected in the UK, from 8 major cities during 2010/11 with associated information such as temperature of storage, country of production, durability etc. All samples were tested for the presence and enumeration of *L. monocytogenes* as well as other Listeria species. All samples were tested at the end of shelf life and duplicate samples of smoked and gravad fish were also tested on receipt as well as at the end of the shelf-life. All food samples were also tested for water activity and pH values. Isolates of *L. monocytogenes* were identified, further characterised and archived. Of the 1,600 samples 400 soft/semi-soft cheeses, 400 heat treated sliced meats and 400 smoked fish (and 400 smoked fish at the end of shelf life).

L.monocytogenes was recovered from 16 (1.3%) samples. *L.monocytogenes* was detected in 4 sliced meats and 12 smoked fish samples, but no samples of soft/semi soft cheese. The levels of *L.monocytogens* detected were all <100 cfu per g except for one sample of sliced meats where the bacterium was detected at 900 cfu per g, enforcement action was taken as a result of this sample.

Materials and Methods

Collection of samples

A total of 400 soft and semi-soft cheeses, 800 hot and cold smoked and gravad fish (not frozen), and 400 heat-treated meat products were collected from 8 major cities (London, Belfast, Cardiff, Glasgow, Birmingham, Leeds, Sheffield and Bradford) in the UK during 2010/11. Samples were collected from major supermarkets reflecting the market share of these products on retail sale. Duplicate samples of each batch of gravad or smoked fish were collected to allow for shelf life testing. The samples (at least 100g) were collected in accordance with the EC Commission Decision (SANCO/5100/2009) from retail outlets and information on country of production, durability date and, instructions on storage temperature were recorded at the point of sampling. The temperature of products at retail display was measured using the infrared sensor of a Combo IR thermometer (Food Safety Direct).

Samples were placed in separate sterile sampling bags and packed in cool boxes with a data logger and sufficient frozen cool packs to maintain a temperature of between 2 and 8°C prior to delivery to the laboratory. Cool boxes were stored at the local laboratories and carried to each sampling location by the sampling officer. The samples were collected as instructed by the sampling plan provided by the Food Standards Agency.

Transport of samples

Transport of samples was carried out by the sampling officer or the preferred provider to the HPA Food Water and Environmental (FW&E) Microbiology Laboratories in Leeds or London by Royal Mail Same Day Delivery. Cool boxes were collected from agreed collection points and transported to the Leeds HPA Food Water and Environmental Microbiology Laboratory by Royal Mail for all samples collected in Belfast and Glasgow. All samples were delivered to the two respective HPA laboratories within 24hrs and at a temperature of between 2 and 8°C and in such a manner to guarantee samples free from external contamination. Tamper evident tags on both the cool box lids and for each individual bagged food sample were used to provide evidence that cool boxes had not been opened during transit. All other samples were transported by the sampling officer to the Leeds or London HPA FW&E Microbiology Laboratories.

Testing of samples

Testing of samples took place in the Leeds and London HPA FW&E Microbiology Laboratories and analysis began within 24 hours of collection for each of the duplicate gravad or smoked fish samples. All other samples were stored in the laboratory at 4 (±2)°C until the end of its shelf life. On receipt, all cool boxes were unpacked by laboratory staff, and temperature profiles saved and examined from the data loggers. All samples were tested for both the presence and enumeration of *L. monocytogenes* using EN ISO 11290-1:1996 and EN ISO 11290-2:1998/Amd1:2004 methods. pH and water activity (a_w) were determined using the methods outlined in EN ISO 2917:1999 and EN ISO 21807:2004 respectively. The identity of one *L. monocytogenes* isolate per sample was confirmed using blood agar, gram stain and the API *Listeria* test kit and all confirmed isolates were sent to the Laboratory of Gastrointestinal Pathogens/ Foodborne Pathogens Reference Unit HPA Colindale for confirmation, typing and storage.

Confirmation and typing of samples

Confirmation of identity, molecular serotyping and typing as well as archiving of *L. monocytogenes* isolates was performed at the Foodborne Pathogens Reference Unit HPA Colindale. *L. monocytogenes* was identified using an in-house duplex real time PCR exonuclease-assay amplifying simultaneously specific fragments of the *L. monocytogenes* haemolysin (hlyA) (Nogva *et al* 2000) and phospholipase A (*plc*A) genes. Molecular serotyping of *L. monocytogenes* isolates was performed using the multiplex PCR assay described by Doumith (Doumith *et al* 2004). *L. monocytogenes* isolates were further characterized by fluorescent amplified fragment length polymorphism (fAFLP) typing following the method developed by Desai (Desai *et al* 2001). Isolates were archived on Cryobeads at -70°C.

Documentation

All procedures were documented using standard operating procedures and data was captured onto a bespoke Microsoft Excel spreadsheet. Statistical analysis was performed using the ANOVA and Chi Squared tests. All procedures were audited against the FSA Code of Practice on Quality Assurance in Research (2003).

Results

Summary

1600 samples were tested in total, these include 400 soft/semi-soft cheeses, 400 sliced meats and 400 smoked fish (and 400 smoked fish at the end of shelf life). *Listeria* species have been detected in 24 (10%) of samples tested. *L. monocytogenes* was recovered from 15 (1.3%) of the samples and was detected in 0 soft/semi-soft cheeses, 4 from sliced meats and 11 from smoked fish. The levels of *L. monocytogenes* detected were all <100 cfu/g except for one sample of sliced meats where the bacterium was detected at 900 cfu/g (Table 1).

Food type	**Number tested**	**Numbers of samples where**	
		***L. monocytogenes* detected**	**Listeria species detected**
Soft/semi-soft cheeses,	400	0	4[i]
Sliced meats	400	4	8[ii]
Smoked fish	400[iv]	11	28[iii]

[i] all *L. innocua*; [ii] 2 x *L. innocua*, 2 x *L. welshimeri*, 4 x *L. monocytogenes*; [iii] 7 x *L. innocua*, 10 x *L. welshimeri*, 11 x *L. monocytogenes*, 5 x *L. seeligeri*, 3 x *L. ivanovii* (seven of the fish samples had more than one Listeria species present)
iv. 400 samples of smoked fish were tested in duplicate on receipt and at the end of shelf life

Table 1: Results of detection of L. monocytogenes and Listeria species in 1600 samples of soft/semi-soft cheeses, sliced meats and smoked fish

Soft / Semi-soft cheeses

400 samples of soft/semi-soft cheeses were tested and no *L .monocytogenes* was detected. *Listeria innocua* was detected in four samples (1% of cheese samples) but at levels of <10cfu/g.

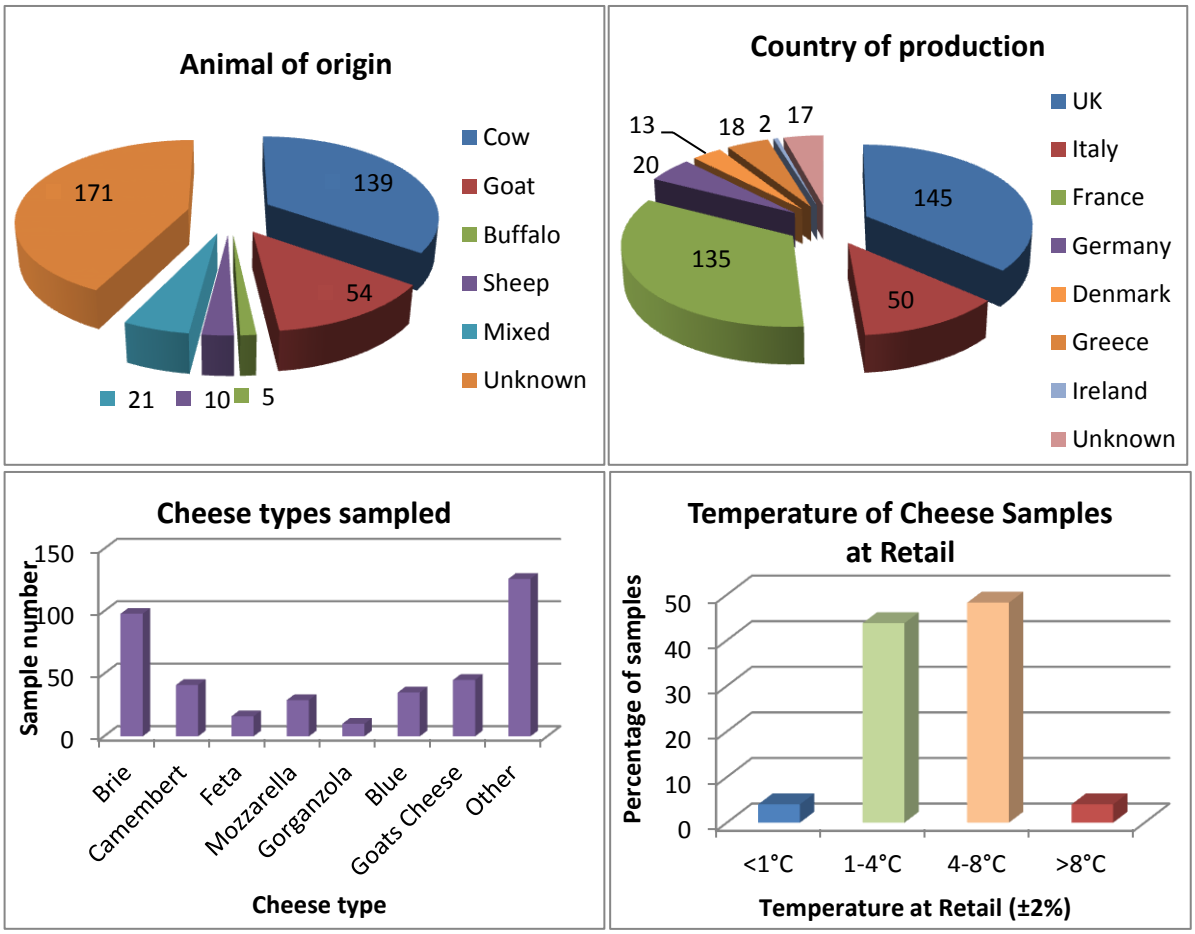

Figure 1: Information on cheese sample details taken at the time of sampling

Where specified, 35% of the cheeses tested were made from cow's milk, 11% goat's milk, and the animal or origin was not specified in the remaining 43%. The largest percentages of samples tested were produced in the UK (36%) and France (34%) and Brie was the most commonly sampled cheese type (25%). The temperature control of the cheese varied between 2 and 9.5 (±1)^{o}C: 4% were found to be above 8^{o}C. The time between day of purchase to end of shelf life varied between 1 and 327 days (mean 26 days, mode 10 days).

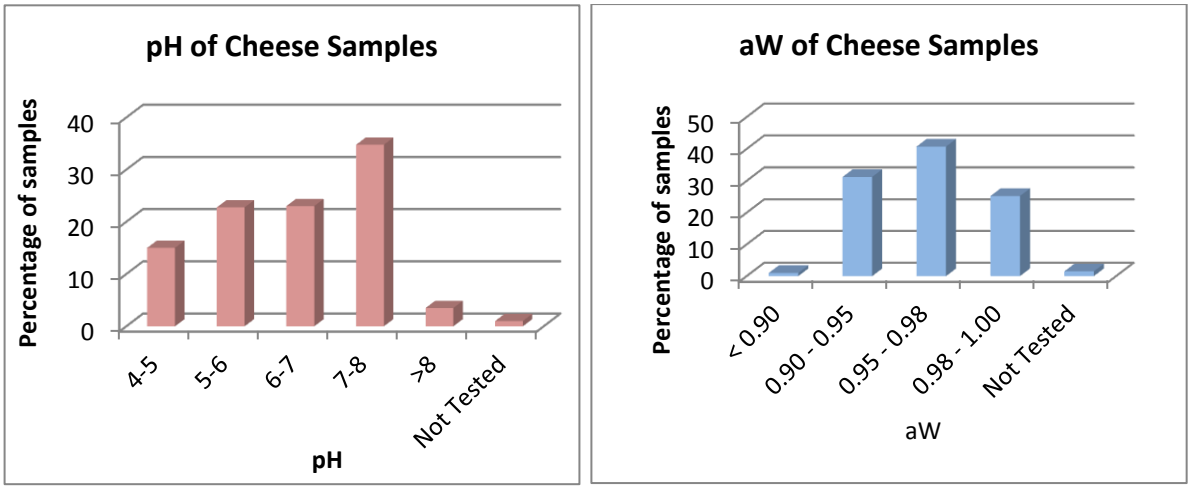

Figure 2: Cheese sample results at the end of shelf-life

The pH range detected varied between 4 and 8.5 with the median/modal pH value of 7.8. The pH range for the four samples where *L. innocua* was detected was 4.5 to 6.7 with three of the samples having a pH below 5. The mean water activity for all the cheese types was 0.96 with most samples having a water activity of 0.98. The water activity for the four samples where *L. innocua* was detected was between 0.93 and 0.98. Using the AVOVA statistical calculation there was no correlation between water activity or pH and detection of *Listeria* species (Chi Squared tests $p > 0.05$).

Heat-treated meat products

400 samples of heat treated meat products that were handling after treatment were tested: all of these samples were sliced products. *Listeria* species (*L. welshimeri*, *L. innocua and L. monocytogenes*) were detected in eight (2%) samples of the sliced meat products at the end of shelf life. *L. monocytogenes* has been detected in four samples (1%) all at <100cfu/g except in one sample (sliced corned beef) where the bacterium was detected at 900cfu/g (the only sample containing levels above the Regulatory limit of 100cfu/g). The time between day of purchase to end of shelf life varied between 2 and 42 days (mean 8 days, mode 7 days).

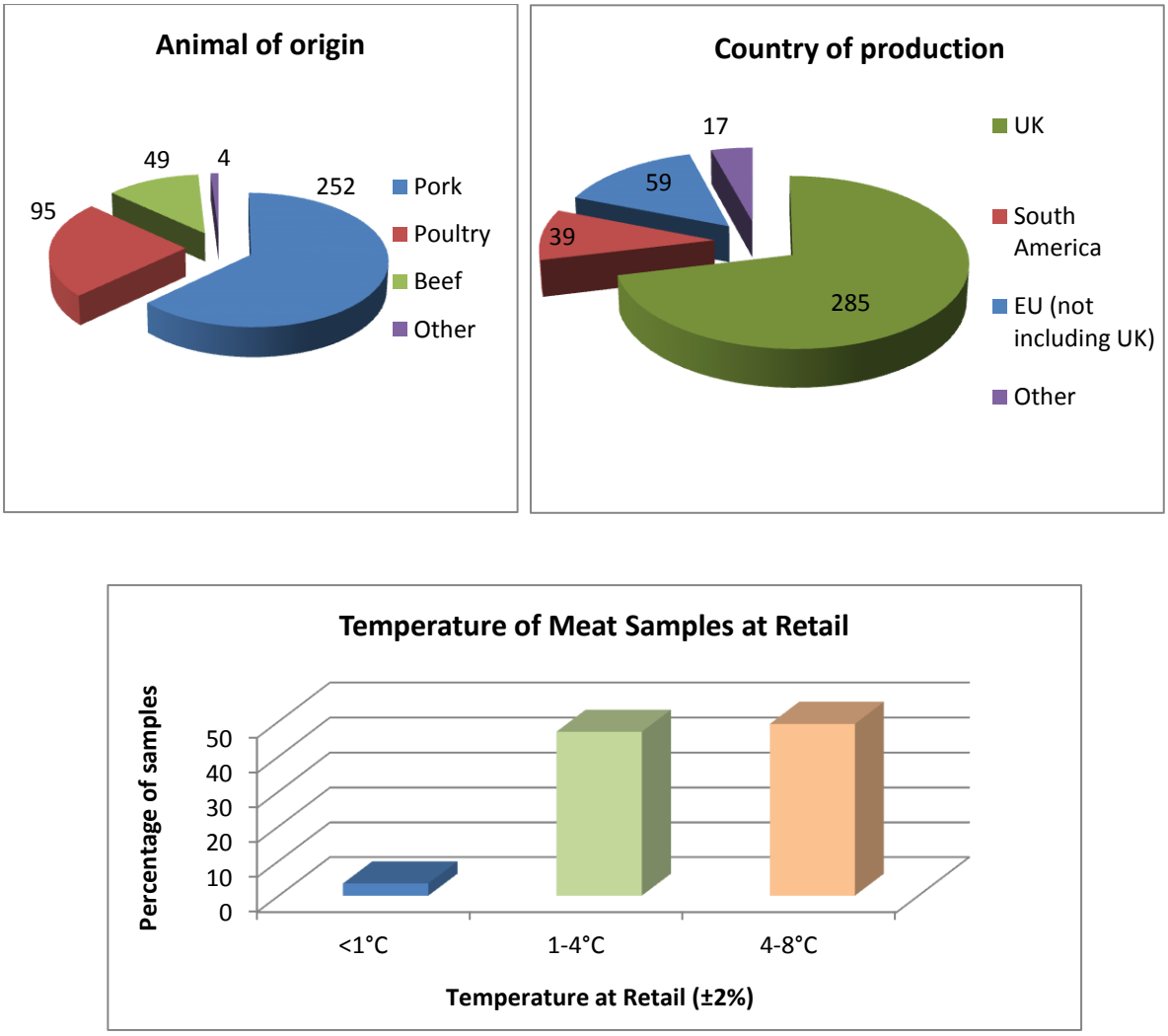

Figure 3: Information on meat sample details taken at the time of sampling

The majority of the sliced meat tested (63%) were ham: the remainder were poultry (24%), beef (12%) or a mixture of animal species (1%) (Figure 3). *71%* of the sliced meat samples tested were produced in the UK (71%). As was found with the cheese samples, temperature control of the sliced meat varied between 2 and 7.5 (±1) ^{o}C: the modal temperature at retail for the meat samples was 4.6 ^{o}C. There was no significant association between the retail display temperature and presence of any *Listeria* species (ANOVA, $p > 0.05$).

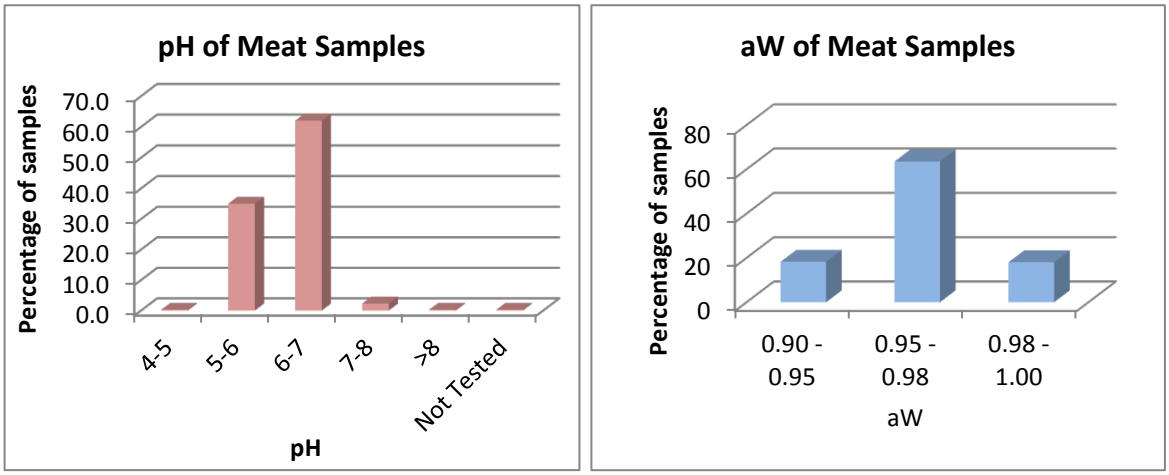

Figure 4: Meat sample results at the end of shelf-life

The pH of the products varied between 5.6 – 8.6, with 3% of samples with a pH of >7 and a standard deviation of 0.4. The water activity varied betweem 0.93 – 1.0, standard deviation: 0.4. There was no significant difference (Chi Squared test, $p > 0.05$) was observed in the pH or water activity for those samples where *Listeria* species was detected. 95% of samples were found to be packaged under modified atmosphere with the other 5% packaged under normal atmosphere: all of the samples where *Listeria* species were detected were packaged under modified atmosphere (ANOVA, $p > 0.05$).

Hot /cold smoked and gravad fish

400 samples of smoked fish have been tested on receipt at the laboratory and 400 duplicate smoked fish samples from the same manufacturer and batch were tested at the end of shelf life. The time between day of purchase to end of shelf life varied between 1 and 40 days (mean 9 days, mode 6 days). *Listeria* species (including *L. monocytogenes)* was detected in 28 samples (7% of all fish samples tested). *L. monocytogenes* was detected in 12 samples (3%) (on sampling both at receipt and at the end of shelf life). In 11 of the 12 samples where *L. monocytogenes* was detected it was only detected on receipt. The level of *L. monocytogenes* detected in 10 of the 12 samples was less than 10 cfu/g, for one sample, 50 cfu/g was detected on receipt and for a second sample the bacterium was detected at 40 cfu/g on receipt and less than 10 cfu/g at the end of shelf life.

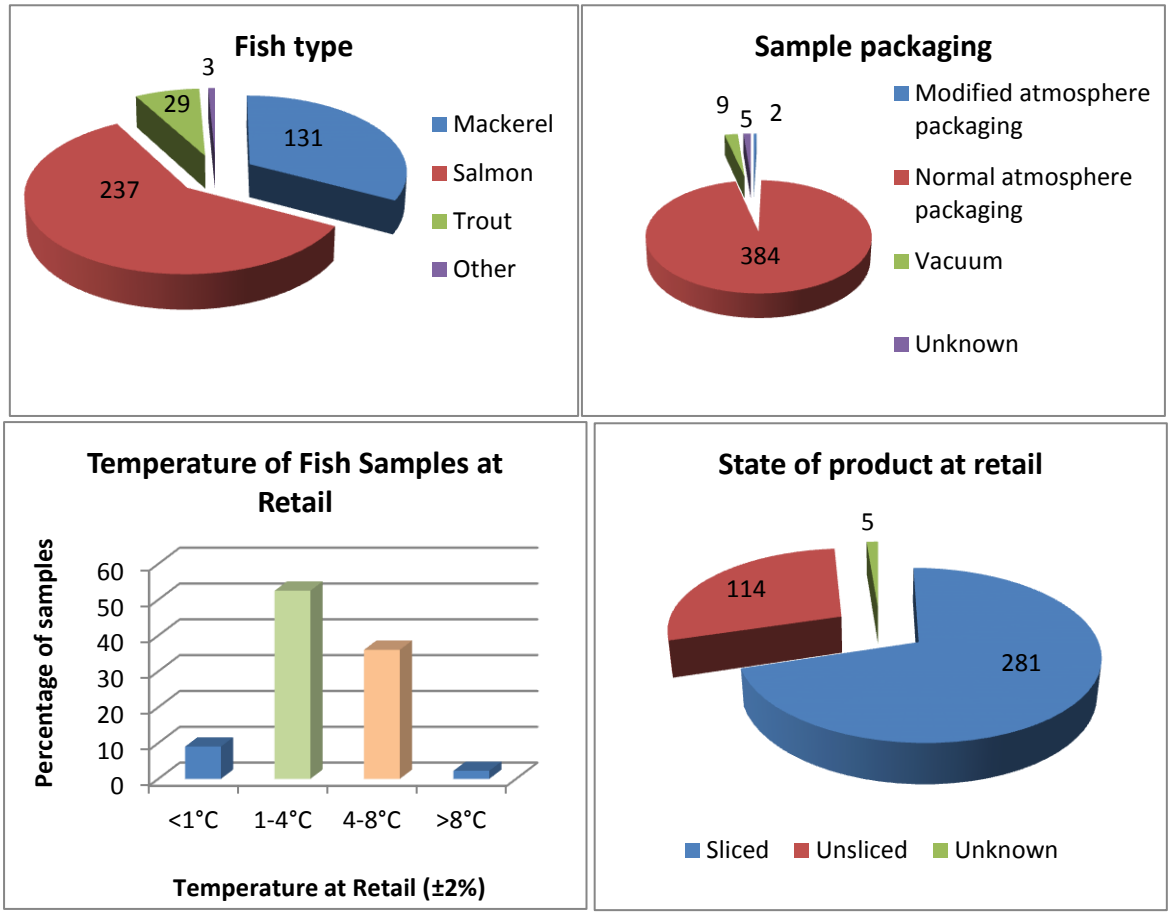

Figure 5: Information on smoked fish sample details taken at the time of sampling

59% of the smoked fish tested were salmon and 94% were produced in the UK. Temperature control of the smoked salmon at display varied between 1.5 and 16 (±1)^{o}C: none of the samples where *Listeria* species were detected had been displayed at above 8^{o}C. 96% of the samples tested were packaged under atmospheric conditions. No trend in the sample surface temperature at retail and the presence of *Listeria* species was observed (the mode is 2.8 ± 1 ^{o}C for all fish samples at retail, while the mode for those fish samples in which *Listeria* species was detected is 4.8 ± 1 ^{o}C). The results shows a higher prevalence (Chi Squared test $p < 0.05$) of *Listeria* species detected in those samples where the fish was sliced and for those packaged under normal atmosphere (although this data may be biased due to the total number of fish samples sliced (70%) or packaged under normal conditions (96%)).

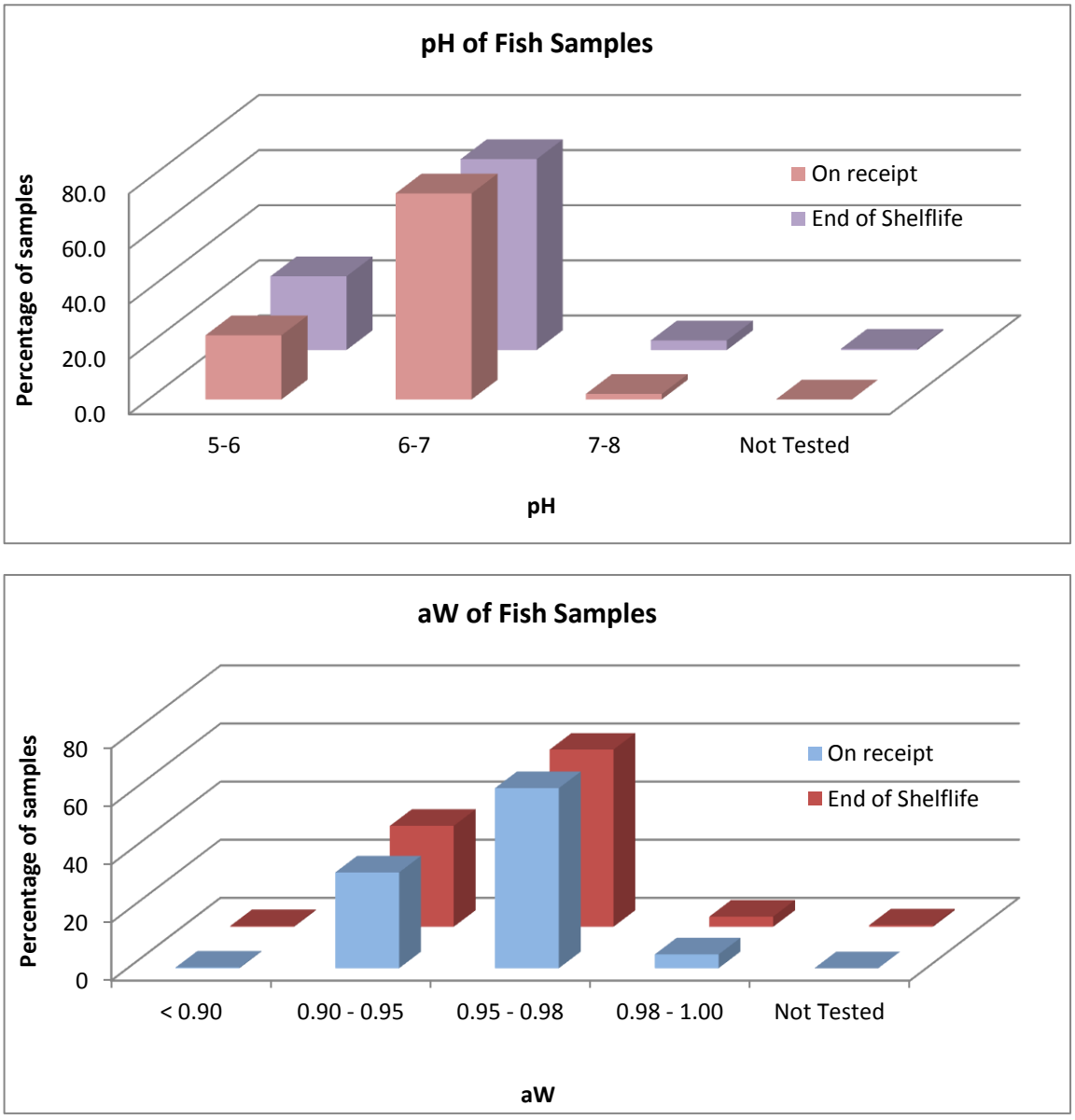

Figure 6: Sample results for the smoked fish products tested

The pH of all the smoked fish products varied between 5.6 and 7.5, and the water activity between 0.9 and 1.0. There was limited variation of both the pH and water activity during storage (pH: mode 6.2, mean 6.1; aW: mode 0.94, mean 0.96) or when tested at the end of shelf life (pH: mode 6.1, mean 6.2; aW: mode 0.96, mean 0.95). There was no significant difference (Chi Squared test, $p > 0.05$) observed in the pH or water activity for those samples where *Listeria* species was detected. While the data does suggest a higher prevalence (Chi Squared test $p < 0.05$) of Listeria species detected in smoked salmon and products produced in the UK this is likely biased due to 94% of smoked fish sampled were produced in the UK.

Analysis of *Listeria* species detected

Listeria species were detected in 40 (4%) of the 1,600 samples tested and *L. monocytogenes* was detected in 15 (1%). This result is similar to the level of *L. monocytogenes* reported in the Zoonoses Monitoring data (EFSA 2009) where *L. monocytogenes* was most often reported from fishery products, cheeses, and meat products at levels of 0.3 % - 1.1 % in the European Union. Furthermore, this survey found that *L. monocytogenes* was only detected above the legal safety limit from ready-to-eat foods in one sample where 900 cfu/g was detected in a sliced corned beef sample.

Table 2: Summary of characterisation results for L. monocytogenes isolates

No	Product	Sample date	Where sampled	EC Code	pH	aw	cfu/g	Species ID	Sero-type	*f*AFLP
1	JOHN WEST,MILD OAK SMKD SCOTTISH SALMON	19.12.10	BIRMINGHAM, B27 6RA	UK AE 009 FE EC	5.94	0.968	<10 cfu/g	*L. monocytogenes*	1/2a	**IX.14**
2	JOHN WEST MILD OAK SMKD SCOTTISH SALMON	20.12.10	LEEDS, LS15 9JA	UK AE 009 FE EC	5.99	0.974	<10 cfu/g	*L. monocytogenes*	1/2a	**IX.14**
3	EX.SPECIAL CHERRYWOOD SMKD TURKEY BREAST	03.01.11	ASDA, BRADFORD, BD18 3RY	UK KM007 EC	6.01	0.991	10 cfu/g	*L. monocytogenes*	1/2c	**VIIc.15a**
4	SMOKED SALMON	03.01.11	MORRISON'S BRADFORD, BD10 8EG	UK AE 006 FE EEC	5.87	0.97	<10 cfu/g	*L. monocytogenes*	1/2a	**IX.14**
5	SCOTTISH SMOKED SALMON	09.01.11	SAINSBURY'S GLASGOW	UK 011FE EC	6.08	0.96	40 cfu/g	*L. monocytogenes*	1/2a	**IX.14**
6	SMOKED SALMON TRIMMINGS	02.05.11	SAINSBURY'S GLASGOW G74 1LX	UK BB 011 EC	5.84	0.96	<10 cfu/g	*L. monocytogenes*	1/2a	**IX.14**
7	6 SLICES COOKED HAM	02.05.11	TESCO GLASGOW G32 6UB	UK K1019 EC	6.06	0.985	<10 cfu/g	*L. monocytogenes*	1/2a	**IX.9**
8	SMOKED SALMON	11.05.11	SAINSBURY'S LONDON NW11 7RX	UK BB011 EC	5.82	0.944	<10 cfu/g	*L. monocytogenes*	1/2a	**IX.14**
9	SMOKED SALMON	11.05.11	SAINSBURY'S LONDON NW11 7RX	UK BB011 EC	6.63	0.928	<10 cfu/g	*L. monocytogenes*	1/2a	**IX.14**
10	WILDWATERS SMOKED SALMON PASTRAMI	17.07.11	TESCO, GLASGOW, G73 1NY	UK SA019 EC	5.93	0.939	<10 cfu/g	*L. monocytogenes*	1/2a	**IX.14**
11	DELICIOUS ESSEX CURED HAM	18.08.11	TESCO CARDIFF CF24 2HP	UK HU200 EC	5.66	0.961	<10 cfu/g	*L. monocytogenes*	1/2a	**IX.22**
12	SALMON TRIMMINGS	18.09.11	MORRISON'S BIRMINGHAM B37 7HR	UK AE006 FE	6.23	0.959	<10 cfu/g	*L. monocytogenes*	1/2a	**IX.14**
13	CORNED BEEF	20.10.11	SAINSBURY'S BELFAST BT11 9AE	UK TC 015 EC	6.34	0.961	**900 cfu/g**	*L. monocytogenes*	4	**I.89**
14	SMOKED SALMON	12.10.11	MORRISONS LONDON NW1 8AA	UK MN039 EC	6.13	0.956	<10 cfu/g	*L. monocytogenes*	**Not Tested**	
15	SMOKED SALMON	12.10.11	MORRISONS LONDON NW1 8AA	UK MN039 EC	6.13	0.963	<10 cfu/g	*L. monocytogenes*	1/2a	**IX.14**
16	SMOKED SALMON	12.10.11	SAINSBURY'S LONDON NW1 9LJ	UK BB011 EC	5.95	0.952	<10 cfu/g	*L. monocytogenes*	1/2a	**IX.9**
17	SMOKED TROUT	04.12.11	ASDA LEEDS LS14 6UF	UK HN039 EC	6.25	0.97	50 cfu/g	*L. monocytogenes*	1/2a	**VI.8**

Sixteen of the 17 *L. monocytogenes* isolated were confirmed as *L. monocytogenes* and all isolates were serotype 1/2a except for two. One of these was serotype 4 and the other was serotype 1/2c. All 16 isolates were analysed by fAFLP molecular typing with 6 different molecular types were identified. The most common fAFLP detected was IX.14 (Table 2). Since the beginning of 2011, *L. monocytogenes* type IX.9, which from this survey was isolated from sliced cooked ham and smoked salmon, has been detected in one clinical case that had a history of eating sliced cooked ham, salmon and haddock.

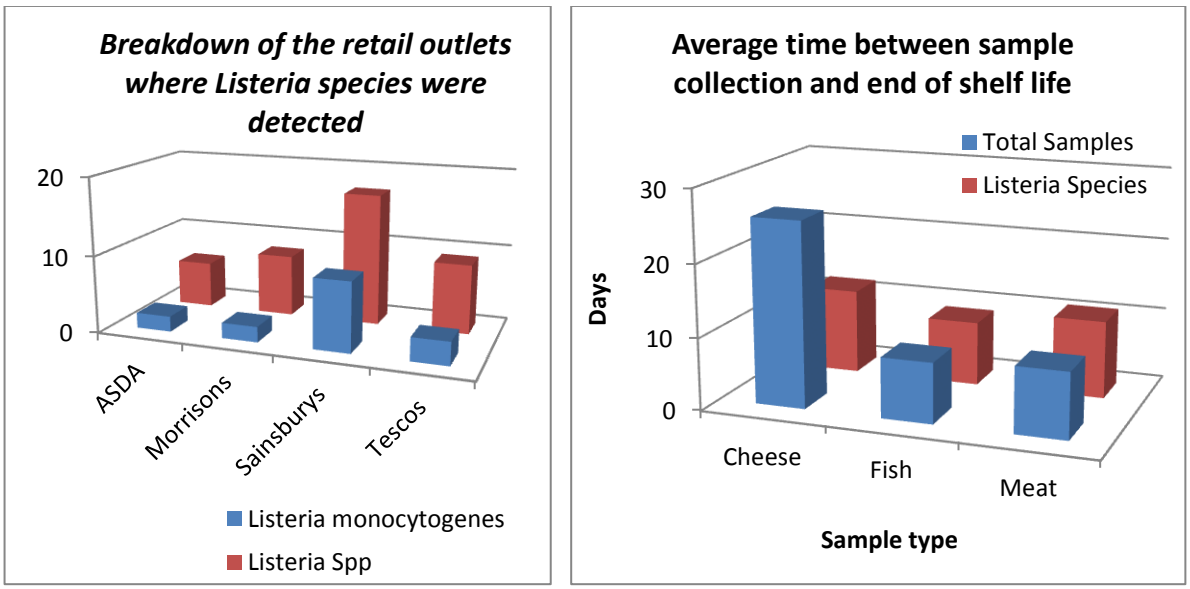

Figure 7: Breakdown of samples where Listeria species were detected

While there was no significant difference (Chi Squared test, $p > 0.05$) between prevalence of *Listeria* species and retail outlets sampled, 43% of samples where *Listeria* species were detected were sampled from the same retailer (35% of *L. monocytogenes* detected). Shop floor storage conditions (temperature, shelf-life etc.) at this outlet did not differ from those of other retail outlets sampled. No significant trend in temperature, water activity or pH and the presence of Listeria species was observed in the samples tested.

Conclusion

Listeria species has been detected in 40 (4%) of the 1600 samples tested and *L. monocytogenes* has been detected in 17 samples (1%). This result corresponds with the reported *L. monocytogenes* contamination rates reported in the Zoonoses Monitoring data as ~ 1% of samples tested (EFSA 2009).

All of the *Listeria* detected was found to be at a level below 100cfu/g, except for one sample where 900 cfu/g was detected in a sliced corned beef sample. No trend in the presence of *Listeria* and storage condition at retail, packaging, country of origin, pH or water activity was observed.

References

Doumith *et al* (Journal of clinical microbiology), Aug. 2004, p. 3819–3822 Vol. 42, No. 8

Desai *et al* (Journal of Clinical Microbiology), Nov. 2001, p. 3823-3829 Vol. 39, No. 11

Nogva *et al (*Applied and Environmental Microbiology), October 2000, p. 4266-4271, Vol. 66, No. 10

The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2009, EFSA Journal 2011;9(3):2090 [378 pp.]