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Validation of the Food (Uncooked comminuted fermented meat) Standard 2008 Under Commercial Conditions

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Scientific Interpretative Summary

This SIS is prepared by MPI risk assessors to provide context to the following report for MPI risk managers and external readers.

Validation of the Food (Uncooked comminuted fermented meat) Standard 2008 Under Commercial Conditions

ESR Report FW11052

Following the introduction of the UCFM standard in 2008, an audit was undertaken of UCFM manufacturers to ensure that the standard was being correctly implemented. In 2010 this was followed up by a survey to evaluate compliance with the microbiological criteria in the *Food Standards Code*. In addition physical characteristics, pH and water activity were measured as these are critical to the production of safe UCFM. Manufacturers were provided with their results and were visited by officials. A follow up survey a year later was conducted. Only the laboratory tests are discussed in this report, so it is not possible to link these with the audit results. This limits the value of the report.

Most of the samples were compliant in both surveys. However pathogens and *E.coli* were found in a few samples. *Listeria monocytogenes* when found was at very low levels in about 10% of samples. Since the study was done, criteria for *Listeria monocytogenes* have been adopted in Standard 1.6.1 of the Food Standards Code and the products tested would have been in compliance. However of concern must be that other *Listeria* species were found in a third of the follow up samples. As these may co-inhabit with *Listeria monocytogenes* it is evident that environmental monitoring for *Listeria* should be adopted by these manufacturers.

The survey confirms that manufacturers of these raw meat products must have a very good understanding of the process and the need and ability to source good quality raw meat. That this understanding is not always the case is evident from one processor whose product showed no evidence of fermentation having occurred, despite their assertion that a culture had been used. It is not known what was found during the official visit to this manufacturer with respect to their use of and understanding of the function of cultures.

From the results it would appear to be important that all manufacturers of these products are regularly audited for compliance with the standard and that they can show from their records that their product is compliant in terms of physical parameters, quality of raw meat and presence of pathogens. Monitoring the environment for *Listeria* is highly recommended. New entrants into the industry should be visited so that any issues can be sorted at any early stage.

Despite the introduction of the UCFM Standard, the results of this study show that these products continue to carry an unacceptable level of risk for children and vulnerable adult consumers.

Client report FW11052

VALIDATION OF THE FOOD (UNCOOKED COMMINUTED FERMENTED MEAT) STANDARD 2008 UNDER COMMERCIAL CONDITIONS

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VALIDATION OF THE FOOD (UNCOOKED COMMINUTED FERMENTED MEAT) STANDARD 2008 UNDER COMMERCIAL CONDITIONS

Prepared for Ministry of Agriculture and Forestry under project MFS/09/04 – Validation of the uncooked comminuted fermented meats (UCFM) standard under commercial conditions, as part of overall contract for scientific services

Client report no. FW 11052

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SUMMARY

The Food (Uncooked Comminuted Fermented Meat) Standard 2008 (UCFM Standard) came into force on 1 December 2008. The standard applies to all UCFM manufacturers whether they are operating under the regime of the Food Act 1981, the Food Hygiene Regulation 1984 or the Animal Products Act 1999. The term UCFM describes a comminuted fermented meat that, during production, has not had its core temperature maintained at 65°C for at least 10 min or an equivalent combination of time and higher temperature. Products manufactured under the UCFM Standard must meet the *Escherichia coli*, *Salmonella* and coagulase positive staphylococci (CPS) microbiological limits of the Australian and New Zealand Food Standards Code 1.6.1 (the Code).

The (then) NZFSA Compliance and Investigation Group (CIG) completed an audit of UCFM manufacturers to ensure that the UCFM Standard was being correctly implemented. Following the audit, the NZFSA supplied ESR with a list of operators so that a retail survey could be conducted to determine the microbiological compliance of UCFM products with the Code under commercial conditions. There were two rounds of sampling. The first tranche took place in 2010 and tranche 2 in 2011. Following the results of Tranche 1, NZFSA wrote to operators and supplied their individual results along with further information on the bacterial hazards associated with the process, and visits from NZFSA officials. Issues identified were difficulties following manufacturing guidelines in accordance to the UCFM Standard 2008, or plant sanitation issues which were seen to compromise the product with regard to the presence of pathogens. The survey was then repeated during 2011 to validate the effectiveness of the UCFM Standard and increased awareness among the UCFM manufacturers.

This report describes the results of a microbiological survey to determine compliance with *E. coli*, *Salmonella* and CPS microbiological limits as specified in the Code. In addition, testing was performed to determine whether samples contained *Listeria monocytogenes* and shiga toxin-producing *E. coli* and, where present, to estimate the concentration of *L. monocytogenes*. In addition pH and a_w measurements were made, and when values for samples were such that growth of *L. monocytogenes* might occur, a fuller chemical analysis was conducted to allow more informed modelling of the fate of *L. monocytogenes* in the product.

In tranche 1 data were obtained from 108 lots of five samples (540 samples tested individually or as 108 pooled samples). Of these, 98.1% (106/108 lots of five) of the UCFM products complied with the Code. Of the two lots that did not comply, one (0.9%) contained *Salmonella* Derby and one (0.9%) had generic *E. coli* counts that exceeded the "m"[1] value of 3.6 MPN g^{-1} in more than one of five samples. In tranche 2, 101 lots of five samples were tested (505 samples tested individually or as 101 pooled samples). Of these 98.0% (98 of 101 lots) complied with the Code. Of the three lots that did not comply all failed the criterion for *E. coli*.

CPS were present at concentrations below "m" for 99.1% of samples. One sample from tranche 1 yielded a count of 2500 CFU g^{-1} which is below the "M" value of 10^4 CFU g^{-1}. In tranche 2 all samples except one contained < 100 CFU g^{-1} and the exception contained 1000 CFU g^{-1}.

[1] Where more than 1 out of 5 individual samples in a lot exceeded an *E. coli* count of 3.6 MPN g^{-1}.

In addition, 30 lots from tranche 1 yielded *Listeria* spp. in at least one sample and, of these, six lots (5.6%) were confirmed as containing *L. monocytogenes*. When *L. monocytogenes* was present the concentration was low, with the maximum recorded being 23 MPN g^{-1}. Among the lots from tranche 2, 38 (37.6%) contained *Listeria* spp., and the prevalence of lots containing *L. monocytogenes* was 9.9%. All positive samples contained the pathogen at <3 MPN g^{-1}. The concentrations of *L. monocytogenes* in positive samples from both tranches were not significant because they were present in a food whose inherent physico-chemical properties do not allow growth of the pathogen.

All of the UCFM lots were tested for the presence of STEC through the detection of the shiga-toxin genes *stx*1 and *stx*2 by multiplex Polymerase Chain Reaction (PCR). Neither gene was detected in tranche 1, indicating that viable STEC carrying either or both of these genes was not present in the enrichment cultures. In tranche 2 one sample was positive for the presence of STEC through the detection of *stx*1, and an isolate of serotype O156:H25 subsequently obtained. This is a potentially pathogenic STEC.

Most (99.5%) lots had both a pH <5.2 and a_w <0.95, values which are considered to define a product on which *L. monocytogenes* will not grow. Some samples did not meet one of these criteria, but when the pH and a_w data were used in the Augustin predictive model none of these produced a "growth' prediction. However, for four samples from tranche 1 the pH of the salami was high and the model predicted growth at 7.7°C. These samples harboured *Listeria* spp., but not *L. monocytogenes*. Two samples had particularly high pH values (6.4 and 6.3) but were of low a_w. In tranche 2 15.8% of samples had a pH value > 5.19, but only one sample also had an a_w exceeding 0.949. The results of further analysis showed that *L. monocytogenes* could grow on this product as determined by predictive microbiological modelling. There was no detectable lactic acid in the single sample from the lot, which suggests that this was not a fermented product, contrary to the information provided by the manufacturer.

There were no differences between tranche 1 and 2 samples for most of the analyses. Lots from both sets of samples were compliant with the Code in 98% of cases. The prevalence of *L. monocytogenes* was higher in tranche 2 samples but was not significantly so. This pathogen was also present at concentrations currently considered to be of no public health significance when present on a food not allowing growth of the organism. The detection of *E. coli* at concentrations in excess of those permitted by the Code indicates that the fermentation step was not sufficient to achieve the required reduction in the concentration of microorganisms of concern with respect to the concentrations present in the raw materials. Of note were the isolations of a *Salmonella* and an STEC, both of which are of public health significance.

1 INTRODUCTION

1.1 Background

MAF has a strategic requirement to reduce the level of foodborne illness in New Zealand. This requires a robust understanding of the exposure of the New Zealand consumer to various pathogens from different foodstuffs, in this case Uncooked Comminuted Fermented Meats (UCFM).

The Food (UCFM) Standard 2008 (the UCFM Standard) applies to all manufacturers of UCFM in New Zealand whether operating under the Animal Products Act 1999, the Food Act 1981 or the Food Hygiene Regulation 1984 regimes, and came into force on 1 December 2008. By definition UCFM is a comminuted fermented meat that, during production, has not had its core temperature increased to, and maintained at, 65°C for at least 10 minutes or an equivalent combination of time and higher temperature. Heat treated versions of salami products are considered as cooked salamis and are not included in this survey. UCFM is generally manufactured from venison, beef or pork that is either of New Zealand or imported origin. Imported pork and pork fat used for UCFM production can only originate from Australia or the EU (Finland). Some UCFM products could contain beef from Australia.

All UCFM must meet the *Escherichia coli*, *Salmonella* and Coagulase positive staphylococci (CPS) microbiological limits as specified in the Food Standards Code 1.6.1[2] (the Code).

Microorganisms	**n[1]**	**c[2]**	**m[3]**	**M[4]**
Coagulase positive staphylococci g^{-1}	**5**	**1**	**1,000**	**10,000**
***E. coli* g^{-1}**	**5**	**1**	**3.6**	**9.2**
***Salmonella* 25g^{-1}**	**5**	**0**	**0**	**-**

[1] n=the number of sample units which must be examined from a lot of UCFM to satisfy the requirements of a sampling plan.
[2] c=the maximum allowable number of defective sample units. When more than this number is found, the lot is rejected by the sampling plan.
[3] m=represents an acceptable level and values above it are marginally acceptable or unacceptable in the terms of the sampling plan.
[4] M=A microbiological criterion which separates marginally acceptable quality from defective quality. Values above M are unacceptable in the terms of the sampling plan and detection of one or more samples exceeding this level would be cause for rejection of the lot.

Further, the UCFM Standard requires manufacturers to meet requirements for *E. coli* as follows:

1. An *Escherichia coli* count for in-going raw meat ingredients used in processing UCFM must be known (to the 98th percentile) and be equivalent to, or below, the process lethality for the validated process.
2. The 98th percentile for an *Escherichia coli* count is determined by using the following:

[2] Available online at (last accessed on 29/11/2011):
http://www.foodstandards.gov.au/_srcfiles/P251%20UCFM%20FAR.pdf

(a) for meat produced in New Zealand:
  (i) the New Zealand National Microbiological Database Programme; or
  (ii) data provided by the company supplying the raw meat ingredients; or
  (iii) data collected by the manufacturer of the UCFM:
(b) for meat imported into New Zealand:
  (i) an overseas data source equivalent to the New Zealand Microbiological Database Programme; or
  (ii) data provided by the company supplying the raw meat ingredients; or
  (iii) data collected by the manufacturer of the UCFM.

3. The number of *Escherichia coli* organisms in UCFM must be measured and recorded (verification) after the product has finished maturation and when the product is ready for sale at a frequency determined by the operator.

The count of generic *E. coli* in raw materials for most domestically processed meat species will generally be determined from New Zealand's National Microbiological Database (NMD). However, there is limited information available nationally on the microbiological quality of meat derived from, or at, other points in the food-chain (e.g. meat processed by butchers or secondary processors), or for imported meat.

Following the introduction of the UCFM Standard on 1 December 2008, NZFSA commissioned ESR to undertake this study, which started in 2010, to validate independently the Standard under commercial conditions, determine compliance with the Code for UCFM, and to evaluate pathogen inactivation. Other than *Salmonella* spp. and CPS, which are included in the code, *Listeria monocytogenes* and Shiga Toxin-producing *E. coli* (STEC) were also included in the analyses as these are pathogens which have either caused outbreaks through the consumption of UCFM products, or could do if present in a sufficiently high concentration.

The project was separated into two streams of work:

(a) An audit of UCFM manufacturers to verify that the UCFM Strandard was being correctly implemented, and a round of microbiological analysis to determine compliance with the Code.
(b) A further round of analyses, tranche 2, of UCFM products "*after the product has finished maturation and when the product is ready for sale*" to determine compliance with the Code under commercial conditions.

The rationale for undertaking such an evaluation is discussed in section 1.2.

1.2 UCFM products and food safety

The UCFM Standard was introduced after the NZFSA assessed data on STEC in raw meat and the level of control achieved by manufacturers (New Zealand Food Safety Authority 2010). The Standard was introduced to ensure that manufacturers control this hazard sufficiently. The organisms selected for consideration were therefore those included in the Standard, STEC because of the prior history of outbreaks, and *L. monocytogenes* because of its importance as a contaminant of ready-to-eat foods and the serious clinical consequences which can result from infection.

A risk profile concerning STEC in UCFM in New Zealand is provided elsewhere[3]. This 2007 report includes an estimate of UCFM production in New Zealand of 343,367 kg, coming 10 producers (Francis Clement, Pork Industry Board, Personal Communication).

The information that follows gives a summary of outbreak data and the prevalence of foodborne pathogens of relevance to New Zealand in UCFM products. This is to establish a context against which the New Zealand data can be compared. More detail is given in Appendix 1.

In general, reported prevalences of *E. coli* O157:H7 in UCFM are low. Because of the lack of data, no comment can be made on the contamination rates for non-O157 STEC in UCFM. Meat and meat products have been associated with STEC outbreaks and incidents overseas but a smaller number of incidents has been linked to consumption of UCFM products.

There have been several outbreaks of salmonellosis arising from the consumption of UCFM products. A recent outbreak of *S.* Montevideo infections in the USA caused 272 cases of disease and was attributed to the pepper used to season Italian style salami (Centers for Disease Control 2010). Examples of other outbreaks include 101 cases in England (Cowden *et al.* 1989), 63 and 83 cases in Italy (Luzzi *et al.* 2007, Pontello *et al.* 1998) and 54 cases in Norway (Emberland *et al.* 2006).

There is little published information to link the consumption of UCFM products and listeriosis. One publication describes an epidemiological investigation into the causes of listeriosis cases in Philadelphia and concluded that salami and ice cream consumption were possible causes of at least some of these cases (Schwartz *et al.* 1989). However, no particular brand could be linked with disease and no consistent subtype occurred in cases. The validity of these conclusions has subsequently been challenged (Louria *et al.* 1990) with the link to the foods attributed to chance at the 95% level because of the large number of foods considered. What is unequivocal is that surveys of UCFM products have reported values for prevalence of *L. monocytogenes* ranging from 0% (Cabedo *et al.* 2008, Thévenot *et al.* 2005) to 45.7% (Petruzzelli *et al.* 2010) and so these foods have the potential to act as the vehicle of cases of listeriosis. To set the risk in context the DoH/USDA quantitative risk assessment assigned "dry/semi-dry fermented sausages" to the "low risk" category on both per annum and per serving basis (Department of Health and Human Services and the United States Department of Agriculture 2003).

The authors of the current report were unable to locate any reports of intoxications caused by CPS in salami. However, these organisms are present in raw meat (Hudson 2004) and so have the potential to grow and produce toxin if the fermentation is inadequate. Their comparative ability to grow at low water activities may extend the proportion of the curing period over which they would be able to grow compared to other foodborne pathogens. CPS have been detected in "dry/semi-dry sausage" with 21 of 2,304 (0.9%) samples containing the organism at a concentration $>10^2$ g^{-1} (Little *et al.* 1998).

[3] http://www.foodsafety.govt.nz/elibrary/industry/Risk_Profile_Shiga_Toxin_Producing_Escherichia-Science_Research.pdf

2 MATERIALS AND METHODS

2.1 Sampling Programme – Tranche 1

A total of 108 lots of UCFM product was tested, with each lot comprised of five units following the requirements of the Code where n=5. The definition of a unit is as follows:

- A whole roll of UCFM (150 g or more) in a vacuum pack;
- Part of a roll of UCFM (150 g or more) in a vacuum pack that is packaged by the manufacturer. Each part must come from a different roll;
- Slices of part of a roll of UCFM (150 g or more) in a vacuum pack that is packaged by the manufacturer. Each pack must come from a separate roll;
- Whole thin sticks of UCFM (i.e. bier more) packed by the manufacturer in a sealed vacuum pack or sealed loose pack weighing 150 g or more.
- Pieces of thin sticks of UCFM packed by the manufacturer in a sealed vacuum pack or sealed loose pack weighing 150 g or more.

Each lot of five units was subjected to analysis of *E. coli* per unit using an Most Probable Number (MPN) 3 rows x 3 multi-tube method, enumeration of CPS by plating on Baird Parker (BP) agar (Hudson 2004), presence/absence testing for *Salmonella* per five unit pool (125 g) using a resuscitation step and two selective enrichment procedures, presence/absence testing of STEC (O157:H7, O26:H11, O145:NM/H-, O103:H2, O111:NM/H-) per five unit pool (125 g), presence/absence testing for *L. monocytogenes* and, if present, the concentration was determined using an MPN method.

2.2 Sampling Programme – Tranche 2

A total of 101 lots of UCFM product were tested, with each lot comprised of five units following the requirements of the Code where n=5. The definition of a unit was as for section 2.1, and each lot was subjected to the same testing regime as those in tranche 1.

2.3 Sample Selection and Preparation

Products from a total of 15 manufacturers, representing all operators, were tested. The intention was to have identical sampling plans between the two tranches, but inevitably there were differences between the two tranches caused by, for example, operators ceasing production. Where there were only a few products per manufacturer one lot of five samples of each product was tested. Where the number of products per manufacturer was large the types tested were prioritised according to criteria such as ingredients (pork, beef and venison, and some non National Microbiological Database[4] (NMD) meats (e.g. kangaroo)), spices (samples collected were prioritised according to potential for contamination and possible inhibitory effects) and sausage size (thick products preferred to thin ones). An over-riding consideration of the sampling plan was to have even representation of manufacturers with respect to the predicted inactivation of *E. coli* in their products according to the University of Tasmania model[5], i.e. >2 $\log_{10}$ reduction, 1-2 $\log_{10}$ reduction and <2 $\log_{10}$ reduction. Above all, it was required that all samples tested had not been heat

[4] http://www.foodsafety.govt.nz/industry/general/nmd/
[5] http://www.foodsafetycentre.com.au/docs/Salami%20Final%20report.pdf

treated and must have gone through a fermentation step by lactic acid-producing bacterial culture in its manufacturing.

The aim was to test whole chubs in their original casing. However, where varieties manufactured by boutique or commercial processors were made and sold as large sausages weighing a kilogram or more and costing $35-40 or more per kg, only short lengths weighing 200-300 g were purchased. Alternatively, vacuum packs of sliced product were obtained from the processors with instructions to ensure the contents of each package came from a different chub (in order to satisfy the n=5 criterion of the Code).

Samples were purchased from supermarkets, boutique delicatessen retail outlets or ordered directly from the manufacturer. In each order, specific instructions were given to ensure that five separate samples from the same lot of products were sampled. Products that were sliced by the manufacturer were vacuum packaged before despatch to ESR by courier in their original unchilled state.

On receipt, each chub or vacuum packaged sample was photographed, and details such as best before, expiry or date of manufacture recorded, and product information examined and recorded to ensure that each was a fermented product and that a cooking step was not included. All samples were stored at 4ºC until required for testing.

Samples were swabbed with 70% alcohol to sanitise the outer casing or packaging. For whole chubs, three 2-3 cm wide portions were then selected (two portions from approximately 2 cm from each end of the chub, and the third from the middle). The casings were removed aseptically from the portions which were then aseptically diced into smaller pieces on a sanitised cutting board with a flamed knife. Sanitation of the entire cutting board surface was achieved by scrubbing with detergent, rinsing under running potable water, drying and disinfection by spraying with 70% alcohol. Excess samples were kept in a sterile bag at 4ºC for further microbiological or chemical testing, if required.

To prepare an homogenate of each sample for *E. coli* MPN and CPS enumeration, a 1:2 dilution was prepared by homogenising 50 g of UCFM in 100 ml of 0.1% peptone diluent in a 24 oz Whirlpak bag (Nasco BO1196WA, Modesto, California, USA). This will be referred to subsequently as the 1/3 sample homogenate.

2.4 *Escherichia coli* MPN

In order to test samples against the standard in the Code (where c=1, m=3.6, M=9.2 MPN g^{-1} for *E. coli*), a three tube MPN method series was required. To prepare a 1/10 dilution, 6 ml of the 1/3 sample homogenate was transferred into 14 ml of peptone diluent to make a 10^{-1} overall dilution. Further dilutions to 10^{-3} were prepared in the same diluent. A 0.1 ml volume of the 10^{-1} dilution was inoculated into each of three tubes containing 10 ml Lauryl Tryptose broth (LST, Merck 1.10266, Darmstadt, Germany). The next two dilutions were then used to inoculate sets of three tubes in the same manner and all nine tubes incubated at 35ºC for 24 h and 48 h at which time the tubes were examined for growth and gas formation. Positive control (*E. coli* NZRM 916), negative control (*S. aureus* NZRM 917) and an uninoculated control were set up in parallel to the test samples.

The presence of *E. coli* in each of the positive LST broth tube was confirmed by sub-culturing a drop of the culture with a sterile orange stick into a tube of EC broth (Difco

231430, Benton Dickinson, Sparks, MD, USA) plus 4-methyl-umbelliferyl-β-D-glucuronide (MUG). These tubes were incubated at 44.5ºC for 24 h and observed for the production of blue fluorescence when exposed to UV light. Positive control (*E. coli* NZRM 916), negative control (*Enterobacter aerogenes* NZRM 798) and an uninoculated control were set up in parallel to the test samples. Tubes showing fluorescence were scored and the results reported as MPN g^{-1}.

2.5 Enumeration of CPS

From the 10^{-1} dilution prepared as described in section 2.4, 0.1 ml volumes were inoculated (in duplicate) onto Baird Parker (BP) agar (Merck 1.05406; supplemented with Egg Yolk Tellurite, BBL 212357, Benton Dickinson, Sparks, MD, USA) plates and spread with a sterile plastic spreader. Positive control (*S. aureus* NZRM 917), negative control (*S. epidermidis* NZRM 1210) and an uninoculated control were set up on BP agar plates.

Plates were then incubated at 35ºC for 48 h. Representative colonies identical to CPS on control plates were selected and tested for the production of coagulase using rabbit coagulase plasma (Pro-Lab Diagnostics, Ontario, Canada). Up to five representative colonies were picked, streaked on sheep blood agar and incubated overnight at 35ºC. Control cultures as described above were set up in parallel. Each isolate was cultured in 2 ml of Brain Heart Infusion broth (BHI, BBL 237500) overnight at 35ºC. To test for coagulase production, 0.5 ml of BHI culture and 0.5 ml rabbit plasma were mixed together in a sterile tube and incubated at 35ºC. Positive (*S. aureus* NZRM 917) and negative (*S. epidermidis* NZRN 1210) and uninoculated controls were set up in parallel. Coagulase activity was monitored after 1 h and 4 h. If negative, the tubes were left for 24 h at room temperature for final observation. Coagulase-producing isolates were scored, counts extrapolated and reported as CFU CPS g^{-1}.

2.6 *Salmonella*

To allow for the recovery of sub-lethally injured *Salmonella* spp. a dual selective broth method was chosen in accordance with ISO6579 (4^{th} Edition), horizontal method for detection of *Salmonella*. Buffered Peptone Water (BPW, Merck 1.07228) is the non-selective pre-enrichment broth and Rappaport Vassiliadis Soya peptone (RVS, Difco 4081) and Muller-Kauffmann Tetrathionate-novobiocin (MKTTn; Oxoid, CM1048, Basingstoke, Hampshire, England) broths were the selective enrichment broths.

A 25 g subsample of UCFM product from each of five units was pooled and stomached for 2 min in 1125 ml of BPW in a Whirlpak Bag (Nasco BO1295WA). The homogenate was allowed to stand for 60 min at room temperature before the pH was adjusted to 6.8 ± 0.2 with sterile NaOH solution, if required, and incubated at 35ºC for 24 h. Volumes of the pre-enrichment were inoculated into each of 10 ml of RVS broth (using 0.1 ml) and 10 ml of MKTTn broth (using 1 ml). The RVS broth was incubated at 42 ± 0.2ºC for 24 ± 2 h, and the MKTTn broth incubated at 43 ± 0.2ºC for 24 ± 2 h. A loopful of each selective enrichment culture was streaked onto one plate each of Xylose Lysine Deoxycholate (XLD, Merck 1.05287) agar, Hektoen Enteric agar (Difco, 285340) and Bismuth Sulfite agar (BS, Difco 273300). All plates were incubated at 35ºC for 24 h.

Following incubation, plates were examined for *Salmonella*-like colonies and BS plates re-incubated for another 24 h. Any *Salmonella* like colonies (up to five colonies per plate) were confirmed using the standard laboratory procedure for *Salmonella* identification (Andrews *et al.* 2001). All isolates were submitted to ESR's Enteric Reference Laboratory for serotyping using the Kauffman-White method (Popoff and Le Minor 2001).

2.7 Shiga Toxin-producing *Escherichia coli*

A combination of multiplex PCR (mPCR) to screen for presence of shiga toxin genes *stx*1 and *stx*2 (Paton and Paton 1998) followed by immunomagnetic separation (IMS) on mPCR positive samples was used for STEC detection.

To perform the *stx* gene screen by mPCR, a 25 g subsample of UCFM product from each of five units was pooled and stomached for 2 min in 1125 ml of pre-warmed TSBC+N in a Whirlpak Bag (Nasco BO1295WA). To make 1 litre of TSBC+N broth, 30 g tryptic soya broth (TSB:Merck, 1.05459) and 10 g casamino acids (Difco 223120) were added to 1 l of deionised water, swirled to mix and autoclave at 121°C for 15 min. After autoclaving and cooling, 5 ml of a sterile aqueous solution of 4 mg ml^{-1} novobiocin (Sigma N1628-5g) were added to make the complete enrichment broth.

Samples were incubated at room temperature for 1 h and then at 42 ± 1°C for 18-22 h, and the pH adjusted to 7.4 ± 0.2 at 25ºC, if required. DNA was extracted from 10 ml of enrichment culture using a DNeasy kit (Qiagen Blood and Tissue, 60504, Qiagen, Hilden, Germany) and the remaining enrichment culture kept at 4ºC for STEC isolation if required. Samples were then tested using STEC mPCR (Paton and Paton 1998). This mPCR has been validated for use in a UCFM-enrichment broth for detection of *stx*1 and *stx*2 genes prior to this survey (Brandt and Cornelius 2010).

Samples which tested positive for *stx* genes by mPCR were subjected to IMS followed by culturing for STEC. Individual IMS following the Dynal protocol was employed using Dynabeads® for five STEC serotypes (O157, O26, O145, O103, O111; Invitrogen, Carlsbad, CA, USA). Following IMS, a volume (50 µl) of the Dynabead® suspension was pipetted onto MacConkey (Difco) and CT-SMac (Zadik *et al.* 1993) agars to obtain suspect STEC colonies. Prior to streaking of the inoculum on the agar surface, a sterile cotton bud was used to break up the IMS-Dynabead® clumps. Streaking with a loop was then performed to obtain individual colonies on the selective agars. Plates were also inoculated from the original sample that had not been subject to IMS. The plates were incubated at 42 ± 1°C for 15-22 h. Presumptive isolates were purified and screened by mPCR to identify those that carry the *stx*1 and/or *stx*2 genes. Isolates were kept on dorset egg slopes for eventual serotyping and virulence gene characterisation by the ESR Enteric Reference Laboratory using methods prescribed in the US Overseas Market Access Requirements (Ministry of Agriculture and Forestry 2011).

2.8 *Listeria monocytogenes*

Each lot of five units of UCFM was tested using a qualitative screen per five unit pool (25g from each unit, 125 g in total) following the Meat Industry Microbiology Methods (MIMM) 4th edition, 2008, 7.5 *Listeria monocytogenes* method (MIRINZ 2008). A positive

result from this test was then followed by enumeration of *L. monocytogenes* from individual units in the pool using an MPN method.

Detection was achieved by enriching in Buffered *Listeria* Enrichment Broth (BLEB, Oxoid CM0862). A volume (125 ml) of BLEB (without supplements) was added to 125 g of UCFM and homogenised in a stomacher for 2 min. A further volume (1000 ml) of BLEB was added and the homogenised sample allowed to stand for 60 min at room temperature before the pH was checked to ensure that it was pH 7.3 ± 0.2 at 25ºC. The enrichment broth was incubated for another 3 h at 30ºC, and 5 ml of sterile anti-microbial supplement (SR149A, Oxoid, Basingstoke, England) added prior to incubation at 30ºC for a further 44 h. After 24 h and 48 h of incubation, a cotton swab was used to inoculate a half plate of PALCAM agar (Difco Palcam base 263620 with Difco Palcam antibiotic supplement 263710) and half plate of Agar Listeria Ottaviani and Agosti (ALOA, AES, Chemunex Ltd, Bruz, France) agar and streaked with sterile disposable loops to obtain single colonies. These plates were incubated at 35ºC and examined at 24 and 48 h for characteristic grey-green colonies with a surrounding black precipitate (PALCAM) or blue-green, regular circular colonies with an opaque halo (ALOA). Suspect colonies (up to five per pool) were confirmed by performing Gram stain, catalase, tumbling motility, aesculin hydrolysis and CAMP tests. Two colonies were selected for full identification using the Microgen™ (Camberley, Surrey, UK) *Listeria* ID kit

At the time of isolate confirmation, a 1/10 dilution of the UCFM sample stored at 4ºC was prepared in 225 ml of BLEB for enumeration of *Listeria* using the MPN method (MIRINZ 2008), and homogenised in a stomacher for 2 min. Tenfold dilutions were then prepared in BLEB down to the 10^{-3} dilution. The MPN was set up consisting of 1 ml of the 10^{-1} dilution inoculated into each of three tubes, 1 ml of the 10^{-2} dilution inoculated into each of three tubes and 1 ml of the 10^{-3} dilution into a further three tubes. All tubes were incubated at 30ºC for 1 h, the pH of each tube checked and adjusted, if necessary, to pH 7.3. All tubes were re-incubated all for another 3 h at 30ºC. Oxoid supplement O149A was then added to each of the nine tubes, and the same supplement along with 4 ml BLEB added to the original sample. All tubes and sample were incubated for a further 44 h at 30ºC. After 24 and 48 h incubation, a loopful of culture from each MPN enrichment tube was streaked onto half plates of PALCAM and ALOA agars. The plates were incubated at 35ºC and examined at 24 h and 48 h for characteristic colonies. If present, up to five typical colonies were taken for confirmation. If all tubes were negative, the result was recorded as <3 MPN g^{-1}.

2.9 Chemical analyses and predictive modelling

All composites of five units from each lot were tested for pH and water activity (a_w). Samples falling outside pH <5.2 and a_w <0.95 (parameters considered as preventing the growth of *L. monocytogenes*) were considered as potentially allowing the growth of *L. monocytogenes*. For samples not meeting these limits, or being close to them, the values of pH and a_w were used in a growth/no growth boundary predictive model (Augustin *et al.* 2005). This model was used as a screen as it allows the input of low pH and a_w values. The model uses inputs of temperature, pH (assuming lactic acid is the acidulant), water activity, phenol concentration, nitrite concentration and proportion of CO_2 in the packaging atmosphere to give a prediction as to whether *L. monocytogenes* would grow under those input physico-chemical parameters, or not. The paper gives a range of values (p=0.1 to 0.9)

where the prediction is neither growth nor no growth, and this was termed the "uncertain domain" (Augustin *et al.* 2005).

The temperature of incubation was taken to be both 4°C to represent refrigerated storage and 7.7°C which represents the mean plus one standard deviation of the temperature of New Zealand domestic fridges (Gilbert *et al.* 2007). In New Zealand retail outlets UCFM products are generally sold refrigerated even though they are a shelf stable product.

If the Augustin (2005) model returned a value predicting growth, or one in the uncertain domain further chemical analysis and modeling was performed. The analyses performed included moisture content, water phase salt (%), lactic acid, sorbic acid, diacetate, citric acid, benzoic acid, acetic acid, smoke phenols and nitrite. Total phenols were measured using a spectrophotometric method (Cardinal *et al.* 2004), and nitrite by HPLC (Eggers and Cattle 1986). Salt (NaCl) was measured according to the AOAC (2005) using a Varian AA40 atomic absorption spectrometer (Varian Techtron Pty Ltd, Mulgrave, Victoria, Australia). Benzoic and sorbic acids were measured by steam distillation and High Performance Liquid Chromatography (HPLC) analysis (Woodward et al. 1979). a_w was measured using an Aqualab CX3 (Decagon, Pullman, WA, USA) water activity meter operated according to the manufacturer's instructions. Measurements of pH were done using an Orion Model 230A electronic pH meter (Thermo Fisher, Waltham, MA, USA). Analyses for citric, lactic and acetic acids were performed according to the HPLC method used by Mejlholm and Dalgaard (2009), the details of which have been published elsewhere (Pecina *et al.* 1984). Moisture content was determined according to established methods (Kirk and Sawyer 1991). This allowed calculation of the concentration of the preservatives in the water phase.

Detection limits for the chemical analyses were; water phase salt 0.05% (w/w), benzoic acid 20 ppm, sorbic acid 10 ppm, pH report to 1 decimal place, A_w report to 2 decimal places, moisture to nearest 0.1%, phenol 1 ppm. Lactic and acetic acids could be detected at 9 ppm and quantified at 30 ppm.

In one sample tested in tranche 2 the analysis detected two unidentified interfering compounds that masked the lactic acid HPLC peak and rendered experiments with spiked samples valueless. Since the problem lay with measuring absorbance in the UV-visual spectrum, a less sensitive method which measured refractive index had to be used to determine the lactic acid concentration.

These additional chemical data were used for modelling of growth of *L. monocytogenes*. Two growth/no growth models were used to assess the probability that *L. monocytogenes* could grow in the product. These first model was the Seafood Spoilage and Safety Predictor (SSSP v 3.1), which is available from http://sssp.dtuaqua.dk. This model uses data from all of the parameters listed above, but is limited in its application by the minimum pH accommodated (5.6) and a maximum water phase salt NaCl concentration of 8%. A second model used can operate at lower pH values (Augustin *et al.* 2005) but does not consider many of the parameters accommodated by SSSP.

3.0 Statistical analyses

Where necessary data sets were compared using the two sample Kolmogorov-Smirnov test (Dytham 2011). A value of 0.5 or greater indicates that the two datasets are not different.

3 RESULTS

Data for tranche 1 were produced for 108 lots of five samples (540 samples tested individually or as 108 pooled samples). Of the lots, 106/108 (98.1%) met the microbiological limits specified by the Code. One lot (0.9%) contained *Salmonella* Derby and one lot (0.9%) had *E. coli* concentrations in excess of 3.6 MPN g^{-1} in more than one sample per lot.

Thirty lots (27.7%) of UCFM contained a *Listeria* spp., of which six (5.6%) contained *L. monocytogenes*. Three of these lots contained mixed contamination of *L. monocytogenes* and other *Listeria* spp. However, concentrations of *L. monocytogenes* from individual samples did not exceed 23 MPN g^{-1}.

In tranche 2, 101 lots were tested (505 samples tested individually or as 101 pooled samples). Of these 98.0% (98 of 101 lots) complied with the code. Of the three lots that did not comply all failed the criterion for *E. coli*. One lot contained *E. coli* O156:H25, an STEC, but all samples from the same lot were found to contain *E. coli* <3 MPN g^{-1}.

Thirty-eight lots (37.6%) contained *Listeria* spp., and the prevalence of lots containing *L. monocytogenes* was 9.9%. All positive samples contained the pathogen at <3 MPN g^{-1}.

Detailed results for each hazard or indicator are presented below.

3.1 *Escherichia coli* concentrations

3.1.1 Tranche 1

E. coli counts equal to or greater than 3.6 MPN g^{-1} were detected in ten samples belonging to six lots. Four manufacturers were associated with these six lots. One lot of these six, a set of five bierstick samples (*E. coli* counts of <3 (1/5), 3.6 (2/5), 9.2 (2/5) MPN g^{-1}), did not comply with the generic *E. coli* criterion of the Code.

3.1.2 Tranche 2

Fifteen (3.0%) of the 505 values exceeded 3.6 MPN g^{-1}. Three (3.0%) of the lots did not comply with the Code with respect to *E. coli* concentration. All non-compliant batches came from one manufacturer; one gave *E. coli* MPN g^{-1} values of 43 (1/5), 23 (3/5) and 9.2 (1/5), another 9.2 (2/5), 23 (2/5) and 15 (1/5) and the last <3 (3/5), 9.2 (1/5) and 23 (1/5).

The overall distribution of MPN values is shown in Figure 1. It can bee seen that the distributions of concentrations measured was very similar for both sets of data. While relatively high concentrations were measured in tranche 2 they were rare.

3.2 CPS

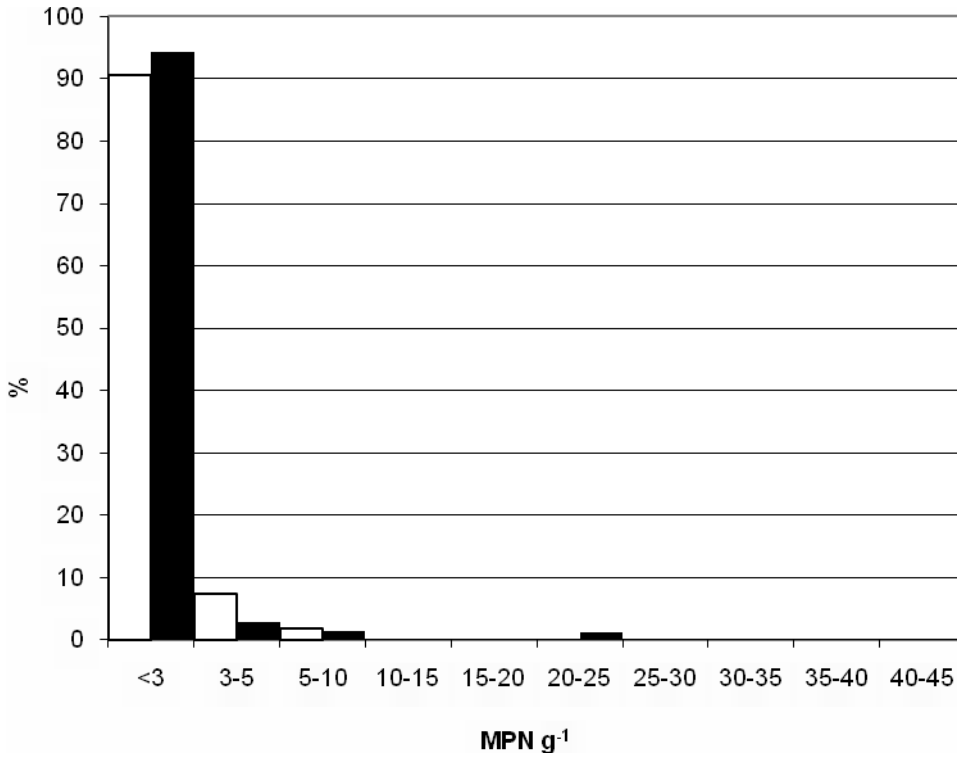
3.2.1 Tranche 1

CPS were detected at low concentrations in 13 samples belonging to eight lots. Nine samples had counts of 1.0 x 10^2 CFU g^{-1}, and one each with 2.0 x 10^2, 2.5 x 10^2 and 3.5 x 10^2 CFU g^{-1}, and one sample with 2.5 x 10^3 CFU g^{-1} (Figure 2). The Code permits one unit out of five per lot (the "c" value) to contain a CPS concentration of up to 10^3 CFU g^{-1}, but none must exceed 10^4 CFU g^{-1} ("M" value). Therefore all samples complied with the CPS limit in the Code.

3.2.2 Tranche 2

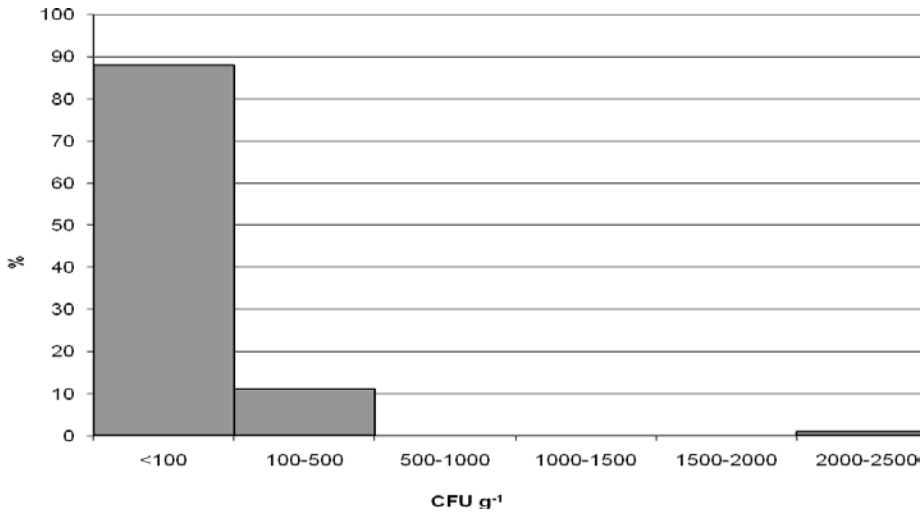
In this tranche, all samples except one contained CPS at < 100 CFU g^{-1}, and the one exception yielded a concentration of 10^3 CFU g^{-1}. All samples therefore complied with the Code. The data for the combined dataset are shown in Figure 3.

Figure 1. Distribution of MPN values for *E. coli* in all UCFM samples



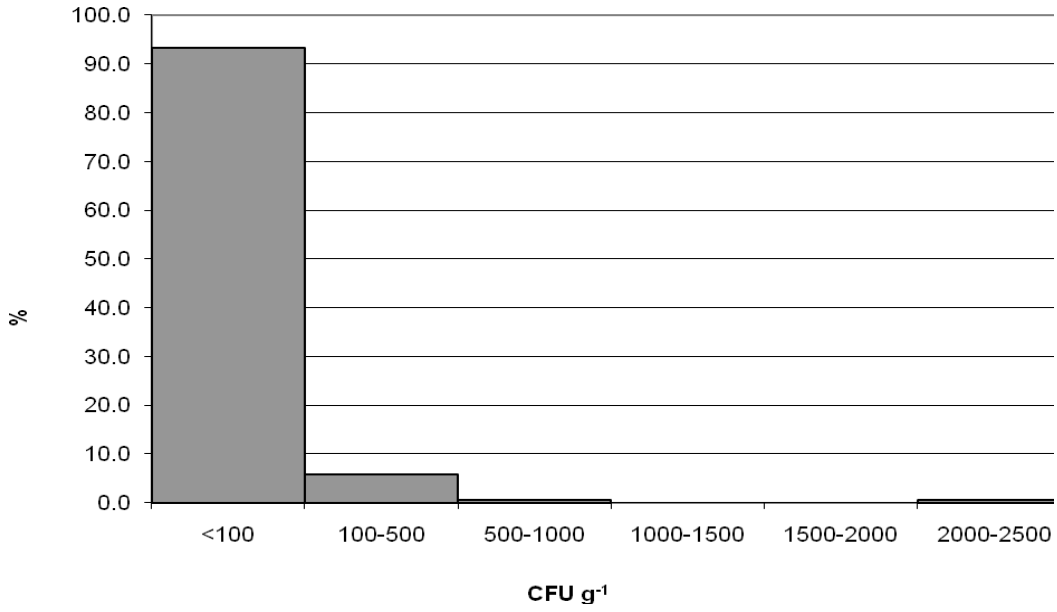
White bars are samples from tranche 1 and black bars those for tranche 2.

Figure 2. Distribution of CPS concentrations in 540 UCFM samples from tranche 1.



3.2.3 Combined data for both tranches

Figure 3. Distribution of CPS concentrations in all UCFM samples.



3.3 Shiga toxin-producing *Escherichia coli*

3.3.1 Tranche 1

All of the 108 lots of five pooled samples were negative for the presence of shiga-toxin genes *stx*1 and *stx*2 by multiplex-PCR, indicating that viable STEC carrying either of these genes was not present in the enrichment cultures of the UCFM samples.

3.3.2 Tranche 2

Of the 101 pooled samples, only one was positive for STEC in the PCR screen. This was a lot of five wild goat salamis (approximately 200g each) purchased from Manufacturer A. A composite of the five samples was analysed qualitatively on the same day for *Salmonella* and *Listeria*. Individual samples were also quantitatively analysed for generic *E. coli* and CPS. *Salmonella* and *Listeria* spp. were not detected. STEC was detected by PCR and *E. coli* O156:H25 isolated in culture. This STEC carried the *stx*1, *eae*A and *hly*A virulence genes. Generic *E. coli* enumerations from the five samples were <3 MPN/g. Four of five samples produced a count of <100 CFU/g CPS while one sample produced a count of 1000 CFU/g.

Subsequently, five more samples of goat salami of the same batch were obtained from Manufacturer A in a follow-up investigation by the NZFSA. In the laboratory investigation, each salami was divided into quarters. The two ends of the salami were combined to form one sample and a second sample was made from the remaining centre portion (1/2 of the roll). Full details of the sampling are given in Appendix 2.

Each roll was separated into outer end, end core, middle outer and middle core samples. Altogether 20 samples were analysed for STEC by PCR, as well as pH and a_w. The results are summarised Table 1 below. All samples tested negative by multiplex PCR. This indicated that STEC including *E. coli* O156:H25 were not present in the salami enrichment culture.

The salami was prepared from meat from feral animals processed in regulated facilities. NZFSA assisted the manufacturing facility with their investigation of any likely cause. Processing and sanitation procedures were in accordance with expectations for the sector, although the premises had not determined the *E. coli* count of the feral raw material and therefore was unable to determine an appropriate inactivation requirement as per the UCFM Standard.

Table 1. Summary of the PCR screen and physicochemical results for salami samples from the same batch as that containing STEC O156:H25.

Date tested	Both Ends of Salami						Middle part of Salami					
23/05/2011	STEC Outer	a_w	pH	STEC Core	a_w	pH	STEC Outer	a_w	pH	STEC Core	a_w	pH
Salami 1	ND	0.743	4.59	ND	0.746	4.59	ND	0.749	4.65	ND	0.75	4.63
Salami 2	ND	0.743	4.63	ND	0.748	4.59	ND	0.749	4.65	ND	0.75	4.68
Salami 3	ND	0.749	4.62	ND	0.762	4.6	ND	0.757	4.65	ND	0.757	4.65
Salami 4	ND	0.753	4.68	ND	0.756	4.66	ND	0.748	4.71	ND	0.747	4.66
Salami 5	ND	0.753	4.59	ND	0.753	4.59	ND	0.752	4.66	ND	0.747	4.65

ND = STEC Not detected

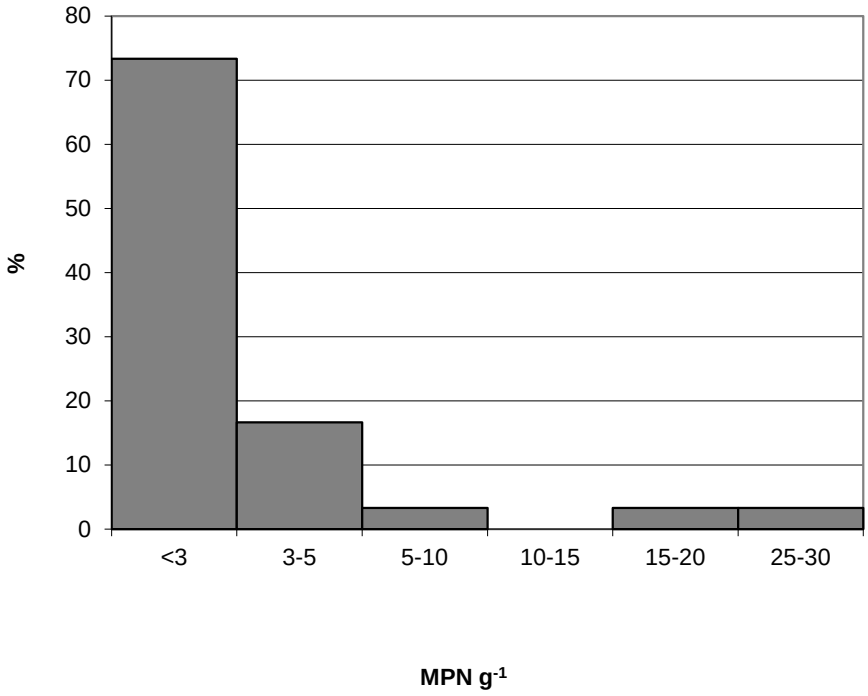
3.4 *Salmonella* spp. and *L. monocytogenes*

3.4.1 Tranche 1

One lot of five samples from the 108 pooled samples tested positive for *Salmonella* Derby, and so did not comply with the Code. This sample was also contaminated with *L. monocytogenes*, with a value of 3.6 MPN g^{-1} *L. monocytogenes* recorded for one of the five individual samples from this lot. One sample also yielded an *E. coli* concentration of 3.6 MPN g^{-1}. These results prompted an official intervention at retail, leading to a recall of products. Subsequent sampling of a second lot of the same salami did not result in the isolation of *Salmonella* spp., *L. monocytogenes* or *E. coli*.

Thirty (27.8%) lots yielded *Listeria* spp. in at least one sample and of these six lots (5.6%) were confirmed as containing *L. monocytogenes*. Three of these samples also contained *L. innocua*. Data for 30 MPN estimations for *L. monocytogenes* were available (Figure 4), and in all cases the concentration was <100 MPN g^{-1}. A concentration of 100 CFU g^{-1} is used to separate acceptable from unacceptable lots for foods in which growth of *L. monocytogenes* will not occur (Luber 2011), and is prescribed in Annex II of the Codex Alimentarius Commissions "Guidelines to the Application of General Principles of Food Hygiene to the Control of *L. monocytogenes* in Ready-To-Eat Foods-CAC/GL 61-2007"[6]. The highest concentration found was 23 MPN g^{-1}, and the same lot also included two other samples with values of 3.6 MPN g^{-1}, with the two other samples containing <3 MPN g^{-1}.

Figure 4. Distribution of *L. monocytogenes* MPN values in 30 individual samples of UCFM from tranche 1.



[6] http://www.codexalimentarius.net/download/standards/10740/CXG_061e.pdf

3.4.2 Tranche 2

No sample from tranche 2 was positive for *Salmonella*.

Thirty-eight lots (37.6%) were positive for the presence of *Listeria* spp., and of these ten pooled samples (9.9%) were positive for *L. monocytogenes*, although in none of the MPN measurements for individual samples did the concentration equal or exceed 3 MPN g^{-1}. Three lots (3.0%) contained *L. monocytogenes* alone, seven *L. monocytogenes* and other *Listeria* spp., and 28 contained non-*L. monocytogenes Listeria* species.

3.4.3 Comparison of tranche 1 and tranche 2

The low prevalence of *Salmonella* in the two sets does not allow for comparisons to be made.

While the prevalence of *Listeria* spp. and *L. monocytogenes* appears to be higher in the tranche 2 samples, when the confidence intervals are considered there is an overlap and so the prevalence rates are not significantly different. For *Listeria* spp. the confidence intervals were 19.6%-37.2% and 28.2%-47.8% for tranche 1 and 2 samples respectively. For *L. monocytogenes* the equivalent values were 2.1%-11.7% and 4.9%-17.5%.

When both sets of data are combined the prevalence rates were 7.7% for *L. monocytogenes* and 32.5% for *Listeria* spp.

3.5 Chemical analyses

3.5.1 Tranche 1

Of the lots tested 22 (20.4%) had a pH value >5.19, while only one (0.9%) of lot had an a_w ≥0.949. While some lots did not meet one of the criteria, when the pH and a_w data were used in a predictive model (Augustin *et al.* 2005) most of these produced a "no growth' prediction. However, for three lots the pH of the salami was high and the model predicted a result in the "uncertain domain" at 7.7°C. A fourth lot was included as, while a no growth prediction was obtained, the values were very close to a sample where an "uncertain domain" prediction was obtained. These four exceptions were the only ones which were subjected to additional chemical analyses and the results are shown in Table 2.

3.5.2 Tranche 2

Of the lots tested 16 (15.8%) had a pH value >5.19, while only 3 (3.0%) of samples had an a_w ≥0.949. One lot had a pH of 5.2 and an a_w of 0.964 which does not meet the criterion of needing to have pH<5.2 and a_w<0.95. The Augustin model predicted a result in the "uncertain domain" and so this was tested further with additional chemical measurements and then the data subjected to predictive modeling. The results for the one sample from tranche 2 which were subjected to additional chemical analyses are shown in Table 3 and indicate that *L. monocytogenes* would grow in this product if it were present.

3.5.3 Comparison between tranches

Figures 5 and 6 show the distributions of pH and a_w measurements for respectively for both tranches. It is apparent that the results were very similar and statistical analysis confirmed this as the p values produced from the Kolomogorov-Smirnov test were 0.5195 and 0.1026 for the comparison of distributions for pH and a_w respectively.

Table 2. Chemical analysis of four tranche 1 high pH samples of UCFM and the results of predictive modelling.

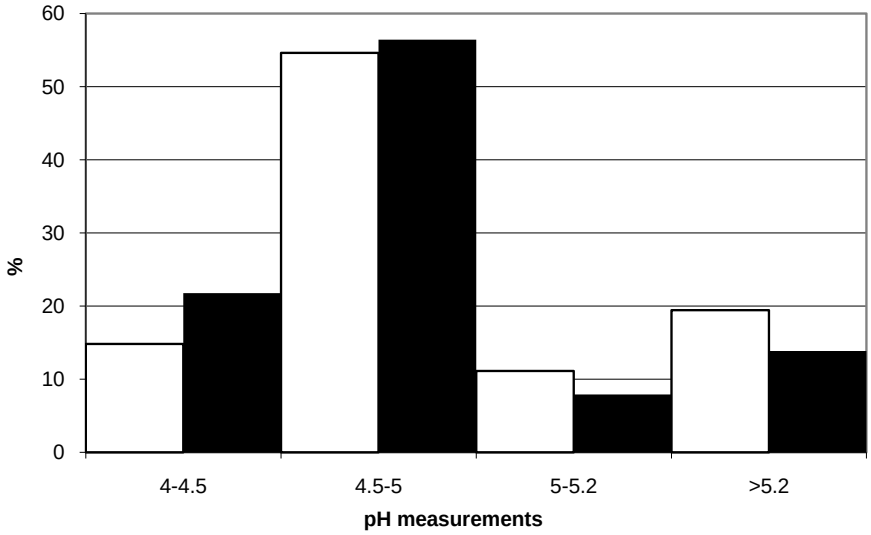
Test	Sample Number			
	1	2	3	4
Screen pH	6.4	6.3	5.4	5.5
Screen a	0.923	0.923	0.941	0.92
Re-measured pH	5.6	5.3	5.2	5.4
Re-measured A_w	0.92	0.92	0.94	0.94
% moisture	50.2	51.3	40.6	42.7
Water phase salt	8.00	6.76	8.21	8.47
Nitrite (ppm)	80	80	110	92
Lactic acid concentration (ppm water phase)	28,315	29,555	42,850	45,005
Acetic acid concentration (ppm water phase)	BDL	BDL	BDL	BDL
Sorbic acid concentration (ppm water phase)	BDL	BDL	BDL	BDL
Citric acid concentration (ppm water phase)	BDL	BDL	BDL	BDL
Benzoic acid concentration (ppm water phase)	BDL	BDL	BDL	BDL
Phenol concentration	21	20	16	19
SSSP prediction (4°C)	NG	NG	N/A	N/A
SSSP prediction (7.7°C)	NG	NG	N/A	N/A
Augustin prediction (4°C)	NG	NG	NG	NG
Augustin prediction (7.7°C)	NG	NG	NG	NG
Zuliani prediction (4°C)	NG	NG	NG	NG
Zuliani prediction (4°C)	NG	NG	NG	NG

N/A: Not applicable, outside the range of the model.
NG: No growth.
BDL: Below detection limit; for water phase salt 0.05% (w/w), benzoic acid 20 ppm, sorbic acid 10 ppm, pH report to 1 decimal place, A_w report to 2 decimal places, moisture to nearest 0.1%, phenol 1 ppm. Lactic and acetic acids could be detected at 9 ppm and quantified at 30 ppm.
NB Maximum concentration of lactic acid accommodate by SSSP is 20,000 ppm and so this value was used. Predictions were made using re-measured values.
Atmosphere assumed to be 0% added CO_2. Diacetate concentration assumed to be 0 ppm.

Table 3. Chemical analysis of one sample of UCFM from tranche 2 with high pH and water activity and the results of predictive modelling.

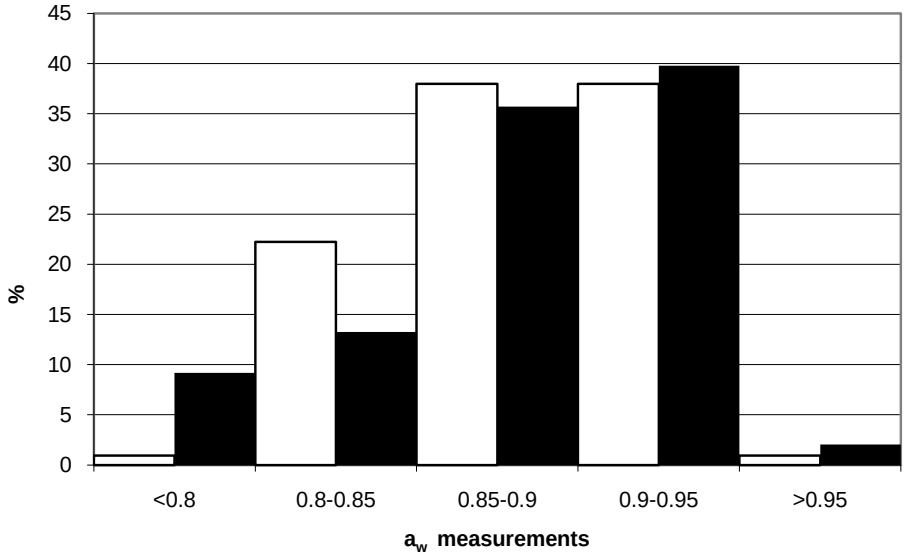
Test	Result
Screen pH	5.2
Screen A_w	0.964
Re-measured pH	6.8
Re-measured A_w	0.964
% moisture	54.0
Water phase salt	5.35
Nitrite (ppm)	60
Lactic acid concentration (ppm water phase)	<500
Acetic acid concentration (ppm water phase)	<500
Sorbic acid concentration (ppm water phase)	<10
Citric acid concentration (ppm water phase)	<500
Benzoic acid concentration (ppm water phase)	<20
Phenol concentration	6
SSSP prediction (4°C)	G
SSSP prediction (7.7°C)	G
Augustin prediction (4°C)	G
Augustin prediction (7.7°C)	G

Figure 5. Comparison of the distribution of pH values obtained for UCFM samples from both sets of samples



White bars are samples from tranche 1 and black bars those for tranche 2.

Figure 6. Comparison of the distribution of a_w values obtained for UCFM samples from both sets of samples



White bars are samples from tranche 1 and black bars those for tranche 2.

4 DISCUSSION

Overall, 97.6% of the samples met the microbiological reference criteria given in the Code, and the presence of *L. monocytogenes* should be considered to be of low risk to the consumer since it was present at low concentrations in a food whose physico-chemical properties suggest that growth of the organism is unlikely, and inactivation the more likely outcome. CPS were present at concentrations well below those at which toxin production is likely to occur and so pose negligible risk to the consumer. However, the detection of STEC and *Salmonella*, even at the low prevalence rates and concentrations recorded, is of concern. Both of these groups can cause disease when consumed in low numbers in a small proportion of exposures.

In tranche 1 *Salmonella* Derby was isolated from one lot of five UCFM samples. The same sample also yielded *L. monocytogenes* at a low concentration (<3 CFU g^{-1}). This was a New Zealand pork-based product which also contained spiced oil, but it may not have been either of these ingredients which were contaminated. For example, contaminated red and black pepper was the source of an outbreak of *S.* Montevideo in the USA in 2009 (Centers for Disease Control 2010). Elsewhere in the world, the presence of *Salmonella* is rare in UCFM products (Table 5, Appendix 1) but when present, can cause disease and outbreaks (Table 6, Appendix 1). It is possible that the *S.* Derby contaminant could have been present in raw pork and survived at a low concentration in the finished product. Follow-up testing of retail samples from the same lot of product did not yield any more positive samples. *Salmonella* was not detected in any of the 101 lots tested in tranche 2 of the survey.

In overseas surveys, the prevalence of *E. coli* O157:H7 in UCFM products is very low at 0-3.3% (Table 7, Appendix 1). However, there can be serious clinical consequences when STEC (*E. coli* O157:H7 and non *E. coli* O157 serotypes) contaminate UCFM products (Table 8, Appendix 1). In 1995 in South Australia there was a mettwurst associated outbreak in which one person died and many others suffered from haemolytic uremic syndrome (23 cases), thrombotic thrombocytopenic purpura (3 cases) and gastrointestinal symptoms (105 cases). As a result FSANZ revised the UCFM processing Standard in Australia in 2003 (Section 1.6.2 of the Code, Australia only). The introduction of the UCFM Standard 2008 in New Zealand was "developed because when the NZFSA completed an assessment of the existing data and information on the pathogen, shiga-like toxin producing *Escherichia coli*, found in raw meat used in UCFM products, it identified that the current level of control during production of UCFM products, in some premises in New Zealand, was insufficient to assure safety.[7]"

STEC were not detected in any of the lots tested in tranche 1. However, in tranche 2 a single STEC was isolated; STEC O156:H25 was isolated from a composite of five samples of wild goat meat salami. It was present at a very low concentration, as the generic *E. coli* counts were all <3 MPN g^{-1}, and was possibly not even present in every roll. It can also be concluded that even though a sample may meet the Standard for *E. coli* it may contain STEC at very low concentrations. In a follow-up investigation a further five samples from the same batch of goat meat UCFM were analysed, and *E. coli* O156:H25 was not detected, even though four subsamples of each salami were tested. It can further be concluded that either the STEC was absent in this second set of goat salami, that the extra

[7] http://www.foodsafety.govt.nz/elibrary/industry/guidelines-production-uncooked-guide/food-standards2008.pdf

storage time (two months) has rendered them non-viable, or that they were present at such a low concentration that they were unable to be detected in the weight of sample tested. Follow up investigations were conducted by NZFSA staff and actions were taken by the operator.

E. coli O156:H25 can be isolated from cattle. For example, It was isolated from a rectal sample from Canadian feedlot cattle being fed a growth promoter (Diarra *et al.* 2009). The isolate tested positive for the *eae*A and *hly*A genes, but not *stx*1. A total of 32 isolates was obtained from three German cattle farms (Geue *et al.* 2010). This serotype has also been isolated from healthy dairy goats (Cortés *et al.* 2005) and sheep (Blanco *et al.* 2003). In these reports the serotype was regarded as an enteropathogenic *E. coli* (EPEC). This serotype was included in a list of those STEC classified as "caused other illness", with such illness being classified as either mild diarrhoea, bloody diarrhea, abdominal pain, ulcerative colitis, haemorrhagic colitis or thrombotic thrombocytopaenic purpura (Hussein 2007). It can therefore be concluded that the detection of this serotype in a food was of public health significance in that it could have caused disease. Serotype O156:HNM (H Non-Motile) was isolated from an animal (no further details available) in New Zealand in 2008.

For this study Tryptic soy broth + casaminoacids (TSBC) + n, without modification, was used as a result of laboratory error as the broth base for the enrichment medium for STEC analyses instead of m-TSBC+n. The difference between the two media is that m-TSBC + n contains 2.5 g l^{-1} bile salts and 1.5 g $^{-1}$ more di-potassium hydrogen phosphate than m-TSB+n.

Because the nomenclature for these media is confusing (for example TSB is sometimes used to represent a medium that is actually TSB plus supplements). Table 4 has been provided to denote the compositions of the various media used. The media names may not match those used in other studies but they are used for clarity in this discussion.

Table 4. Composition of some media used for the enrichment of STEC

Component	Medium			
	m-TSB+n[1]	TSB[2]	TSBC+n[3]	m-TSBC+n[4]
Tryptone	17.0	17.0	17.0	17.0
Sodium chloride	5.0	5.0	5.0	5.0
K_2HPO_4	4.0	2.5	2.5	4.0
Dextrose	2.5	2.5	2.5	2.5
Soy peptone	3.0	3.0	3.0	3.0
Bile salts No. 3	1.5	-	-	1.5
Novobiocin	20 mg	-	20 mg	20 mg
Casaminoacids	-	-	10	10

[1] Lab M formulation, weights in g/l unless otherwise stated
[2] Merck 1.05459 formulation
[3] As used here
[4] Medium dM TSB-CA (Vernozy-Rozand 1997). This broth was the one originally intended to be used in this study.

The USDA's formulation of m-TSBC + n aims to optimise recovery by inhibiting the growth of contaminating and competing microflora while supporting more rapid growth of *E. coli* O157:H7 on fresh beef. However, unlike fresh beef products, the environment in a UCFM matrix is more hostile to the pathogen. Any STEC present would be acid- and salt-stressed as a result of fermentation and dehydration processes, respectively. The viability of STEC would be affected particularly following a period of maturation (Glass *et al.* 1992). It is likely that the use of TSBC+n instead of m-TSBC+n as the enrichment broth base would offer a slight advantage with resuscitation and growth in the enrichment broth. The advantage of using a medium without bile salts has been shown in work where the addition of 1.5 g l^{-1} of bile salts to m-TSB (with or without novobiocin) recovered fewer acid or salt stressed cells of *E. coli* O157:H7 at 37°C and 42°C compared to recovery in TSB (Stephens and Joynson 1998).

For non-O157 STEC it has been reported that one of four *E. coli* O111:H^- isolates did not grow in m-TSB+n at 37°C or 42°C, but all were able to grow in TSB. All of the other STEC serotypes (O157:H7, O157:H-, O111:H8, O26:H11, O141:H4, O130:H11, O145:H25, O48:H21, O128:H2, O104:H7, O91:H-, O:5:H-) were able to grow in both media (Bayliss 2008). However, it is not clear whether the inhibition of the O111:H- isolate was caused by the presence of noviobiocin or bile salts.

Recent observations from the US indicate that TSBC alone is preferred for the detection of STEC as novobiocin, at 20 mg l^{-1} is known to inhibit *E. coli* O111, O145 and O121 serotypes (M. Koohmaraie, pers com. 22/12/2010). This is supported by prior work which demonstrated that the addition of novobiocin to enrichment broth (TSB in this case) can inhibit or retard the growth of non O157 STEC strains (Kanki *et al.* 2011, Vimont *et al.* 2007).

It is likely that the choice of enrichment broth for screening of STEC in food products will undergo further evaluation by the scientific community before settlement on a formulation acceptable to most products. It was agreed with MAF that TSBC+n was to be the enrichment broth to be used for the screening for STEC in UCFM for tranche 2 samples.

Where *L. monocytogenes* was detected in UCFM samples in the present survey, its concentration was <100 MPN g^{-1}, which is the limit considered acceptable in foods not supporting the growth of the organism by international organisations including the EU and Codex Alimentarius Commission.

One major UCFM manufacturer surveyed in tranche 1 of the study appeared to have an endemic problem as *L. innocua* was detected in 50% of lots tested. *L. innocua* was found in a range of this manufacturer's retail products, from 300 g rolls, to sliced products as well as samples from end sections of whole UCFM chubs (80 mm in diameter). Although *L. innocua* is not pathogenic, it occupies a similar ecological niche to *L. monocytogenes* and is suggestive of the potential presence of the pathogen. The results suggest that there was an endemic contamination problem within the processing environment and that the cleaning and sanitation programme, controls for people and equipment, process controls, product separation controls and/or other controls in this plant were not eliminating this, or possibly other, *Listeria* species.

A few lots (4/209) failed to meet the microbiological criteria in the Code because of non-compliant *E. coli* concentrations. This reflects either a process which is operating correctly but using raw materials containing too many *E. coli*, or an inadequate process applied to acceptable raw materials. *E. coli* acts as an indicator of the potential presence of other enteric pathogens such as pathogenic *E. coli* (e.g. STEC and EPEC) and *Salmonella*.

Eighty-eight percent of samples surveyed in tranche 1 contained CPS at <100 CFU g^{-1}, 11% contained counts of <500 CFU g^{-1} and 0.9% contained counts of 2,500 CFU g^{-1}. The results were marginally improved in tranche 2, where all but one sample contained <100 CFU g^{-1}. These CPS data are quite consistent with those in the literature. *S. aureus* was present in soft Italian fermented salami samples at concentrations of <100 CFU g^{-1} (Aquilanti *et al.* 2007), and in a UK survey reported by Little *et al.* (1998), <1% of samples out of 2304 tested positive for CPS with counts of >100 CFU g^{-1} (Table 9, Appendix 1).

Only four salami products in tranche 1 had pH and a_w values measured in the initial screen which could have permitted growth of *L. monocytogenes* according to the model used. The physico-chemical compositions of these lots were not unusual when compared to data for other products (FAO/WHO 2004). For example water phase lactic acid concentrations cited in FAO/WHO (2004) ranged from around 10,000 ppm (around 100 mM) to 57,600 ppm (around 600 mM) while those measured here were from 28,315 to 45,005 ppm.

When further chemical analyses and pH was assessed against three predictive models the consensus was that growth would not have occurred in these products (Table 2). The Augustin (2005) model has subsequently been modified to include lactic acid concentration as an input parameter (Zuliani *et al.* 2007). Both models predicted no growth. These two models are prone to "fail dangerous" predictions (Mejlholm *et al.* 2010). The SSSP model is not well suited to predictions for UCFM products as they mostly have pH values that are too low and may have lactic acid and salt concentrations that are too high to be accommodated by the model.

The only sample in tranche 2 which was tested for parameters important for growth modelling was of a physico-chemical composition (pH 6.8, a_w 0.964) that both models (Augustin and SSSP) predicted would allow growth of *L. monocytogenes*. This sample lacked any measurable organic acids and so it seems likely that it was not a fermented product. It was included in the study as the advice from the manufacturer was that it was a fermented product.

The results of this survey were compared to a survey of pH and a_w characteristics of UCFM products carried out in Toronto (Lee and Styliadis 1996). The Canadian paper reported various criteria that defined a safe UCFM product, but with the values used in this report (pH <5.2 and a_w <0.95), 86.4% of the samples from Toronto would have met the criteria compared with 99.5% of lots in the current New Zealand survey.

There were differences in the pH of samples measured at screening and those measured later for the five samples where full chemistry testing was completed. This may have been caused by bacterial or chemical means during storage between the two measurements.

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APPENDIX 1. SUMMARY TABLES FOR THE PRESENCE OF PATHOGENS IN UCFM PRODUCTS

Table 5. Prevalence of *Salmonella* in UCFM products from overseas surveys

Country	Year	Products tested	Number tested	% positive	Reference
England, Wales and Northern Ireland	1996	Dry/semi-dry sausage	2,304	0.09	(Little *et al.* 1998)
Italy	2007	Salami	NS	0	(Aquilanti *et al.* 2007)
Spain	-	Traditional fermented sausages	19	0	(Martin *et al.* 2011)
Spain	1998-2004	Salami	15	0	(Cabedo *et al.* 2008)

NS=Not stated

Table 6. Specific incidents of disease reported for *Salmonella* associated with UCFM products

Location	Serotype	Year	No. affected	No. deaths	Source	Reference
Denmark	Typhimurium DT193	2010	20	0	German pork and venison salami	(Kuhn *et al.* 2011)
England	Typhimurium DT124	1989	101	0	German salami sticks	(Cowden *et al.* 1989)
Germany	Goldcoast	2001	44	0	Raw fermented sausage	(Bremer *et al.* 2004)
Italy	Typhimurium DT104A	2004	63	-	Pork salami	(Luzzi *et al.* 2007)
Italy	Typhimurium PT 193	1995	83	-	Salami	(Pontello *et al.* 1998)
Norway	Kedougou	2006	54	1	Danish style salami	(Emberland *et al.* 2006)
USA	Typhimurium	1995	26	-	Lebanon bologna	(Sauer *et al.* 1997)
USA	Montevideo	2010	272	-	Pepper used in Italian style salami	(Centers for Disease Control 2010)

- Information not stated

Table 7. Prevalence of *E. coli* O157 in UCFM products from overseas surveys

Country	Year	Products tested	Number tested	% positive	Reference
Argentina	2000	Dry sausage (dry-cured salami)	30	3.3	(Chinen *et al.* 2001)
Australia (Western)	1996-1997	Surveillance data of UCFM	>400	0	Cited in (FSANZ 2003)
Australia (ACT)	2001	UCFM, including sliced meats	41*	0	(Cartwright and Rockliff 2001)
England, Wales and Northern Ireland	1998	Dried and fermented meat and meat products	2,304	0	(Little *et al.* 1998)
The Netherlands	1996	Cooked or fermented RTE meats	328	0.3	(Heuvelink *et al.* 1999)
USA	1995 - 1999	Dry and semi dry fermented sausages	3,445	0	(Levine *et al.* 2001)

*Other meat product samples were included in this study and it could not be ascertained from the paper how many samples of UCFM were tested, only that 41 samples were tested for *E. coli*. It is unclear whether serotype O157:H7 was specifically tested for.

Table 8. Specific incidents of disease reported for STEC associated with UCFM products

Location	Serotype	Year	No. affected	No. deaths	Source	Reference
Australia (South)	O111:H-	1995	23 HUS (one death), 30 bloody diarrhoea, 3 adults with TTP, 105 other GI symptoms	1	Mettwurst	(Centers for Disease Control 1995)
Australia (Western)	O157:H7	2001	2 (1 hospitalised)	0	Cacciatore (pork)	(FSANZ 2003)
Canada	O157:H7	1998	39	NS	Genoa Salami (naturally fermented)	(Williams *et al.* 2000)
Canada	O157:H7	1999	6 HUS; 143 ill (42 hospitalised)	0	Hungarian style sausage	(MacDonald *et al.* 2004)
Denmark	O26:H11	2007	20	0	Fermented organic beef sausage	(Ethelberg *et al.* 2009)
Germany	O157:H- (sorbitol +ve)	1995	28 children with HUS, estimated 300-600 other people	3	Mortadella and teewurst*	(Ammon *et al.* 1999)
Italy	O157	2004	2	0	Pork meat salami	(Conedera *et al.* 2007)
Norway	O103:H25	2006	17, 10 HUS	NS	Cured mutton sausage	(Schimmer *et al.* 2008)
Sweden	O157:H7	2002	30, 9 HUS	0	Cold smoked fermented sausage	(Sartz *et al.* 2008)
USA	O157:H7	1995	20 (including 4 hospitalised and 2 HUS)	0	Presliced deli dry-cured salami	(Centers for Disease Control and Prevention 1995)
USA	O157:H7	2011	14, 3 hospitalised	0	Lebanon Bologna	(Centers for Disease Control 2011)

NS=Not Stated. * Mortadella is a cooked product, but teewurst is substantially fermented and dried. The latter product was considered to be the likely vehicle in this outbreak.

Table 9. Prevalence of *L. monocytogenes* in UCFM products from overseas surveys

Country	Year	Products tested	Number tested	% positive	Reference
Brazil	-	Italian and Milanese style salamis	81	7.4	(Borges *et al.* 1999)
Brazil	2006-2007	Sliced vacuum-packed salami	130	6.2[1]	(Martins and Leal Germano 2010)
Canada	2001	Fermented sausage	100	4.0	(Bohaychuk *et al.* 2006)
France	-	Finished sausages	30	0	(Thévenot *et al.* 2005)
Italy	-	Fermented sausages tested after 1 2 3 4 weeks of ripening	246	 23.2 10.0 22.0 13.0[6]	(Manfreda *et al.* 2007)
Italy	2007	Salami	NS	0	(Aquilanti *et al.* 2007)
Italy	2007-2009	Vacuum-packaged sliced salami	112	20.5	(Di Pinto *et al.* 2010)
Italy	2003-2004	Fermented sausage	237	15.2[2]	(De Cesare *et al.* 2007)
Italy	-	Salami	140	45.7[4]	(Petruzzelli *et al.* 2010)
Spain	-	Traditional fermented sausages	19	15.8[5]	(Martin *et al.* 2011)
Spain	1998-2004	Salami	15	0	(Cabedo *et al.* 2008)
Turkey	2004-2005	Traditional fermented sausage (sucuk)	300	11.6	(Colak *et al.* 2007)
Wales	2008-2009	Fermented meats	316	1.9[3]	(Meldrum *et al.* 2010)

[1]Present at up to 1.9 x 10^3 CFU g^{-1}.
[2]66.7% of samples < 3 MPN g^{-1}, 33.3% 3-9 MPN g^{-1}
[3]Present at <20 CFU g-^{1}.
[4]16/48 +ve 10-7.6 x 10^3 CFU g^{-1}, remainder <10 CFU g^{-1}.
[5] < 100 CFU g^{-1}.
[6] <3 MPN g^{-1} after 4 weeks ripening.

Table 10. Prevalence of CPS in UCFM products from overseas surveys

Country	Year	Products tested	Number tested	% positive	Reference
United Kingdom	1996	Dried and fermented meat products	2,304	0.9*	(Little *et al.* 1998)

*Present at $>10^2$ CFU g^{-1}

APPENDIX 2. DETAILED METHODOLOGY FOR RE-SAMPLING SALAMI FROM MANUFACTURER A

The aim of the investigation was to study the distribution of the STEC in each of the five salami samples. In addition, water activity and pH measurements from the various portions used for STEC analysis was obtained to inform the chemical conditions.

The casing was not tested and was removed aseptically after surface sanitation with 70% alcohol before the salami was divided. The procedure to cut the various portions of the salami is described in the procedure below.

- Sanitise the surface of salami with 70% alcohol. Place salami on a clean board (**Board 1**). Cut off the tie and string from both ends of salami
- Peel casing off salami ensuring that the hand (with glove on) holding onto the cased salami is not used to hold onto unwrapped salami once the casing is removed.
- Place unwrapped salami on **Board 2**. Use a clean knife to cut into 3 pieces (1/4 at each end and ½ in the middle). Transfer ends to **Board 3** and place the cut ends face down on the board. Leave the middle portion on the **Board 2**.
- **Board 2**. Stand middle portion of salami on one end. Use a clean knife to slice outer layers (about 0.5 cm thick) round core. Keep outer slices separate. Transfer core to a whirlpak bag (1 Middle Core). Transfer outer slices to another whirlpak bag (1 Middle Outer).
- **Board 3**. Use a sterile knife to trim outer layers of each end including the upper end areas (about 0.5 cm thick) round core. Keep outer slices separate. Transfer core to a clean whirlpak bag (1 End Core). Repeat for the other end and transfer core to bag (1 End Core). Transfer outer slices to bag (1 End Outer).

After the first salami, use another set of boards and knives to operate on salami 2.

- Salami 2 2 Middle Core, 2 Middle Outer, 2 End Core, 2 End Outer.
- Salami 3 3 Middle Core, 3 Middle Outer, 3 End Core, 3 End Outer
- Salami 4 4 Middle Core, 4 Middle Outer, 4 End Core, 4 End Outer
- Salami 5 5 Middle Core, 5 Middle Outer, 5 End Core, 5 End Outer

- Once all salamis are dissected, weight 25 g sample of each dissected part in a whirlpak bag and enriched as described in the methods section.

Weigh 15g of each sample and place in a volumetric tube for pH and a_w measurements.