DAFF LABORATORIES, BACKWESTON

Newsletter

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NRL Contacts

Antimicrobial Resistance Zoonoses (salmonella)
Dr M Gutierrez

Listeria Staphylococci Milk & Milk Products
Ms B Hickey

Ecoli (VTEC)
Dr L Scott

Parasites
Dr T Murphy

TSE's
Dr P Collery

Residues/Chemical Elements
Mr P Rafter

Pesticide Residues
Dr D O'Sullivan

Campylobacter
Dr J Egan

Animal Proteins
Mr G Roe

Activities of National Reference Laboratories (NRL's)

Introduction

In 2006 following the designation of a number of additional Community Reference Laboratories (CRL's) by EU, Member States were required under Article 33 of Regulation 882 / 2004 to designate one or more National Reference Laboratory (NRL) for each CRL. The Departments of Health and Children and Agriculture and Food, as the Irish Competent Authorities, assigned these NRL functions to a number of laboratories including those within the Backweston Laboratory Campus. See list of NRL's outlined in Appendix.

In this issue:

(a) Report on the 11th Annual Workshop, Community Reference Laboratory on Milk

(b) Report from the 3rd Annual Workshop, Community Reference Laboratory on Campylobacter

(c) Report from the 3rd Annual Workshop, Community Reference Laboratory on E. coli

Also in this issue is a report on the 2nd meeting of the Codex Alimentarius *AdHoc* Intergovernmental Task Force on Antimicrobial Resistance (TFAMR). Stakeholders are encouraged to read this report (full report available on Codex website) as AMR is an issue of growing concern worldwide. Comments may be submitted to Dr John Egan CVRL.

Eleventh Annual Workshop of the Community Reference Laboratory on Milk, Vienna on the 9th and 10th October 2008.

NRL Representative: Bernadette Hickey *(DSL, Backweston Campus)*

The workshop on Milk was dedicated to the topic Phosphatase.

1. Setting Limits for Alkaline Phosphatase Activity in milk from species other than bovine. DG Sanco requested the CRL to examine the possibility of setting phosphatase limits for milk from species other than bovine. The EU limit for bovine milk is already set at 350mU/L of phosphatase activity units.

1.1 Milk from Goats and Ewes

At the 10th Workshop Member States (MS) were requested to check the phosphatase level in milk from goat's and ewes in their own country. Laboratory pasteurisation of the milk was to be carried out in accordance with the protocol forwarded by the CRL. It was thought that the limit for goat's milk could be set at the same limit as for bovine milk. MS reported their findings to the CRL. The results were presented and it was not possible to set a limit as the milk from goats in Romania, Cyprus and Greece would not meet the limit. DG Sanco is to consider how one can implement a limit based on the results. The levels of phosphatase in milk from ewes after pasteurisation was very variable across MS and no limit could be set.

2. Suitability of using Phosphatase in Milk from Mares and Camels.

2.1 Mares Milk

Mare's milk is enjoying a revival and there are equine dairy farms in Belgium, France, Netherlands and Norway. Many therapeutic qualities are attributed to mare's milk. It is usually sold raw at 7-8 Euro / litre. It is also sold as freeze dried powder and can be used in yogurt and ice cream. The level of alkaline phosphatase present in the milk is very low (approx. 2,200 mU/L compared to cow's milk at approx. 800,000mU/L) The enzyme is inactivated at a lower time temperature than pathogenic organisms. Small quantities (<0.2%) of contaminated raw milk in pasteurised product can not be detected.

2.2 Camel Milk

The composition of camel milk differs from bovine milk in the composition of the protein and enzymes. It phosphatase content ranges from 14,000-16,000 mU/L. The phosphatase levels drops to approx 4000mU/L after pasteurisation at 63°C x 30 min or at 74°C x 15sec. The temperature has to be raised to 90°C to get a significant drop in the enzyme level but this temperature affects the flavour of the product.

It was concluded that phosphatase is an unsuitable enzyme to use to check for satisfactory pasteurisation in milk from mares and camels.

3.Equivalence of Alternative Methods vs Reference Method

The reference method for the determination of alkaline phosphatase in milk, ISO 11816-1 2006. ISO 22160 2007 is an alternative method based on enzymatic photo activated system method. The two methods do not give equivalence at the limit for cow's milk of 350mU/L. The alternative method gives more positive results in the range 250-500mU/L.

4. Phosphatase Activity in Cheese

The CRL carried out work on a method to determine the phosphatase activity in pasteurised soft cheese excluding blue mould using ISO 11816-2/IDF 155-2 2003 as the method.

(a) Preparation of Samples

The method states that for soft cheese one should use a glass rod to prepare sample and for hard cheese use a potter / ultraturrex. The CRL found that the ultraturrex worked for all cheese types.

(b) Extraction with Alkaline Phosphatase free milk

The method states that AP free milk can be used to extract the phosphatase. The CRL found that the cheese extraction buffer supplied by Advanced Instruments Ltd gave much better extraction and higher values. The cheese buffer is costly. At the low levels of phosphatase activity found in pasteurised cheese there is no significant difference between extraction with AP free milk and Cheese extraction buffer but at higher levels the extraction buffer extracts approx. double the phosphatase.

This ISO standard is currently under revision and the CRL are recommending:

(i) use of an ultraturrex for sample preparation for all cheese types

(ii) use of cheese extraction buffer.

Advanced Instruments are going to release the composition of

the cheese extraction buffer for inclusion in the revised standard.

4.2 Phosphatase Activity in Hard Cheese made from raw milk

Luisa Pellegrino (State University, Milan) gave a presentation on Phosphatase activity in hard cheese made from raw milk. Cheeses such as Grana Padano and Parmigiano Reggiano must be made from raw milk. The Italians have an AP activity of >300,000mU/g in the outermost layer (1cm depth) of cheese for cheeses such as Grana Padano and Parmigiano Reggiano.

The AP activity in these cheeses is low due to the production method and size of cheese. The cheese is made in a vat and the temperature profile for the cooking steps are as follows- 1st cooking 32°C , 2nd cooking 43°C and final cooking 48°C.

The curd is at a high temperature when placed into large moulds and there is also heat development due to LAB fermentation. The effect of the heat and the large mould keep the cheese core at a temperature of 52-56°C for 8-10h. The surface of the cheese cools rapidly. These conditions lead to a low level of phosphatase activity in the core of the cheese.

4.3 Setting Limits for Alkaline Phosphatase in Pasteurised Cheese

The CRL carried out studies on the phosphatase levels in soft cheese (camembert, brie, coulommiers) made from pasteurised milk, thermised milk and μ-filtered milk. They concluded that it was possible to distinguish cheese made from pasteurised milk from thermised milk and μ filtered milk. The CRL propose that an AP activity limit of 5 or 6 mU/g should be set. MS were asked to check the values obtained in their own countries to check if this limit could be met.

5. Reactivation of Alkaline Phosphatase

There is evidence that alkaline phosphatase reactivates in certain products. There has been no research on this topic since the 1970's. There is a lack of biochemical and structural knowledge about the AP in milk to understand and control this phenomenon. The CRL are undertaking a study on this topic.

6. Interlaboratory Study on Alkaline Phosphatase in Cow's Milk

The CRL organised an interlaboratory study on AP activity in bovine milk. 42 samples containing whole milk, semi skimmed milk and skimmed milk were sent to 19 Laboratories. The samples contained 6 levels of Phosphatase in blind duplicates.
The results were satisfactory and the r and R values of the network of laboratories were better than those published in the ISO standard. The values of the network will be introduced into the revised ISO standard.

7. Work Programme for 2008-2009

The CRL outlined its work programme for the next 12 months as follows;

- Proficiency Trial on goat's milk
- Work on setting phosphatase limits for milk from other species
- Revision of ISO 11816-2
- Setting limits for cheese made from pasteurised milk
- Examining phosphatase in other products such as Cream and Ice cream

Third Annual Workshop of the Community Reference Laboratory for Campylobacter, Uppsala, Sweden, 6th - 8th October 2008

NRL Representative: *Margaret Griffin (CVRL, Backweston Campus)*

1. Dr Ari Hörman, (DG SANCO) stated that the baseline study for *Campylobacter/Salmonella* at retail level proposed for 2009 would not be going ahead as there were no monitoring controls in place. He referred to the Codex guidelines for the control of *Campylobacter* and *Salmonella* spp. in chicken meat. Targets are to be considered for the reduction of *Campylobacter* in broilers or other stages of the food chain and microbiological criteria in foodstuffs. These will depend on cost/benefit analysis, availability of control tools etc.

2. Dr Rene Sjogren Hendrikson (Fodevareinstituttet, DTU, DK) gave a short overview of the antimicrobial resistance component of the baseline study on Campylobacter spp. in carcasses from broiler flocks. He outlined the various methodolgies in use by NRL's participating in the survey.

- E-test by AB-biodisk: involves the use of Mueller Hinton agar with 5% sheep blood, direct colony suspension inoculated onto plate by swab and the addition of an E-test strip, which contains a gradient of antimicrobial concentrations. There are

no recommended antimicrobial susceptibility interpretive criteria for this test.

- Agar dilution: is carried out using Mueller Hinton agar with 5% sheep blood to which various concentrations of antimicrobial are added. These plates are inoculated using a multipoint innoculator. This method is approved for four antimicrobials according to CLSI and twelve antimicrobials according to EUCAST. It is regarded as a laborious and time-consuming method.
- Disk diffusion: also uses Mueller Hinton agar with 5% sheep blood, direct colony suspension inoculated onto plate by swab and the addition of disks containing various antimicrobials at set concentrations. This method is of limited use as MIC cannot be determined.
- Micro-broth dilution: is regarded as the "Gold Standard" for MIC determination. It requires the use of a micro titre plate, which contains antimicrobials of varying concentrations. Trek and VetMIC etc produce these plates. The plates are inoculated with Cation-adjusted Mueller Hinton broth supplemented with 5% sheep blood to which a standard concentration of the organism to be tested is added. The plate is inoculated using a multichannel pipette or automated inoculator. It is approved for four antimicrobials according to CLSI and twelve antimicrobials according to EUCAST. It is an expensive method but easy to use.

Epidemiological cut off values should be used for interpretation of MIC values and EFSA have recommended using the Eucast cut off values. Quality control is important with particular emphasis on the quality of saline, broth, lysed blood, McFarland standard, temperature and microaerobic conditions. The purity of the test suspension for each isolate should be checked. Dr Hendrikson recommended the www.antimicrobialresistance.dk website for further information.

3. Alastair Thomas (Oxoid Ltd., UK) gave a presentation on the development of Brilliance Campy Media, a new medium for the enumeration of *Campylobacter*. It will be available in 2009.

4. Dr Jaap Wagenaar (WHO/OIE) outlined the activities of WHO/OIE, including reference testing for strains, courses, research, and supports available for developing countries. He summarized the methods available for *Campylobacter* typing i.e. serotyping, PFGE, AFLP and MLST. He mentioned the need for exchange of data between labs e.g. Pulse-Net and the MLST database.

5. Dr Ingrid Hanson (CRL) presented results from Ring Test No. 3 undertaken in May 2008 and involved the detection, confirmation, speciation and enumeration of *Campylobacter* spp. from 14 samples. 31 NRL's participated in the ring test.
23 NRL's correctly detected *Campylobacter* in the ring test using both selective media. 3 NRL's correctly detected *Campylobacter* using either selective media, while 8 NRL's failed to detect *Campylobacter* in the correct samples.

NRL's had the option of using phenotypic or PCR methods to speciate the Campylobacter isolates. 14 NRL's used PCR methods for speciation. 22 NRL's correctly speciated the Campylobacter isolated in the ring trial by either phenotypic or PCR methods. In general, identification by PCR gave more correct results compared to phenotyping in the study. The species that caused most problems to correctly identify was Campylobacter lari.

Results from 31 NRL's from the enumeration of Campylobacter in all 14 samples were available. Of the 31 NRL's, 23 correctly reported the correct results on the 14 samples by direct plating on mCCDA for enumeration. Most of the NRL's found a three-log difference between the samples with high and low dose of C. jejuni and C. coli, respectively. The majority of the NRL's started the analyses two days after the samples were dispatched from the CRL. However, no significant difference was found in the amount of Campylobacter spp. between the samples that were analyzed one, two or three days after they had been dispatched from the CRL.

The interlaboratory comparison study demonstrated the usefulness and importance of comparing the overall performance of the NRL's when carrying out these types of analyses, which are similar to the ongoing EU baseline study on Campylobacter in broilers. The information can be used for making improvements of laboratory practices in order to obtain harmonised and optimal performance of analyses in laboratories participating in official control of Campylobacter in EU.

6. Dr Elina Lahti (CRL) gave a short presentation on the estimation of Measurement of Uncertainty (MU) and the results received so far from the NRL's. The MU used in the baseline study is based on ISO/TS 19036 and Commission Decision 2007/516/EC. A global approach is used for the estimation of MU rather than a step-by-step approach in the case of microbi-

ological analysis of food. It is based on the overall variability of the analytical process and is derived from an experimental estimation of the standard deviation of reproducibility of the final result of the complete measurement process. Three different options for obtaining data to estimate the standard deviation of reproducibility: (i) an intralaboratory study, (ii) an intralaboratory proficiency trial or (iii) intralaboratory standard deviation of reproducibility. This latter option was the one recommended by the CRL for the baseline study as data is derived from one laboratory but at different time periods, different analysts, different media batches, reagents and incubators as part of a routine procedure. They recommended testing 12 naturally contaminated samples in parallel between May and September.

Problems encountered included:

- Naturally contaminated samples contained few or low levels of organisms for estimation.
- Time delay between analyses may cause decrease in count during storage.
- Some differences in approach between laboratories.

7. Dr Eva Olsson Engvall (CRL) outlined the quality assurance undertaken by CRL on NRL's participating in the baseline study. A proportion of *Campylobacter* spp. isolates from caecum and carcass samples at each NRL were required to be submitted to the CRL for confirmation and identification. A total of 195 isolates had been received from 17 MS and 2 non-MS prior to this workshop. 9 MS had not yet sent isolates. For 110 / 118 (93%) isolates identification by NRL and CRL corresponded. 4 submitted isolates were mixed *C. jejuni* and *C. coli*. There were low numbers of hippurate neg *C. jejuni* and low numbers of species other than *C. jejuni* or *C. coli* (2 isolates).

8. Dr Marie-José Laisney (NRL-FR) gave a presentation on enrichment plating methods describing the influence of plating media and enrichment period on detection, enumeration and species of *Campylobacter* spp. from naturally contaminated chicken samples. This involved the analyses of 30 chicken leg skins, 30 chicken neck skins and 30 chicken caeca. 12g of these samples were added to 108 mls of Bolton broth and were either plated directly onto selective media, further diluted for enumeration or added to enrichment broth for 24 and 48h enrichment. Selective media used were mCCDA, Butzler, Karmali and Campyfood.

The results were as follows:

- *Campylobacter* enumeration was dependant on medium used with Butzler detecting less *Campylobacter* organisms.
- More positive samples were detected at 24h than 48h due mainly to caeca became heavily contaminated during the longer incbation.
- Direct detection yielded more *Campylobacter jejuni* whereas enrichment was more successful for *Campylobacter coli*.

9. Dr Ria van der Hulst-van Arkel (NRL-NL) gave a presentation on the preparation and results of a ring test undertaken in Holland. The aims of the test were to develop a proficiency testing scheme for *Campylobacter* using swabs, poultry caeca and poultry skin according to the guidelines of ILAC 2000 and ISO/IEC Guide 43-1:1997. The procedure was tested for preparation, homogeneity and stability of test materials during transport and storage up to 8 days. Pure culture swabs, caecal content swabs and chicken skin were inoculated with *Campylobacter* spp. and sent to the 24 participating labs.

In summary all test materials were sufficiently stable and homogenous for the trial when stored after transport up to 8 days.

- 13 labs had correct results for all pure culture samples
- 19 labs had correct results for all caeca samples
- 8 labs had correct results for all skin samples
- 13 labs were allocated performances as good
- 7 labs were allocated performances as sufficient
- 5 labs were allocated performances as unsatisfactory

The proficiency testing scheme for *Campylobacter* will be available for international participation in 2008.

10. Dr John Rodgers (NRL - UK) presented an assessment of the EU survey method for *Campylobacter* detection in broiler caeca. A preliminary media study was carried out using caecal samples on mCCDA, Preston and Skirrow agars. Detection limits were carried out on caecal samples, which were diluted up to 10^7, split into seven aliquots and stored at +4°C. An aliqout of each dilution was tested by direct culture and enumeration every day for seven days. Studies were also carried out on pool sensitivity, enumeration and effect of time on viable numbers.

In conclusion mCCDA and Karmali agar were more sensitive than Preston agar giving detection limits between 10^2 and 10^3 cfu/g while Preston agar showed a much higher limit of 10^5 to10^6 cfu/g. No major drop in levels of *Campylobacter* observed over time. There was some evidence that enrichment selects for

C.jejuni over *C.coli*. Within batchs, prevalence was high for caeca (75% of batches tested were positive). Levels of *Campylobacter* in caeca were high (10^8 cfu/g), in which case a pool of 10 caeca was a sensitive test, even in cases where the pool contained only 1 positive caeca. When negative samples were enriched in parallel with direct culture, a small increase in the number of positive batches was detected. 25 of 27 batches that were tested beyond the 80h deadline were still found positive for Campylobacter.

11. Dr Enne De Boer (VWA, NL) outlined some of the development being undertaken in International Standards. EN ISO 10272-1 for *Campylobacter* detection and Enumeration is due for review probably in 2009. EN ISO 10272-2 Colony Count technique will possibly be revised in 2009/2010. EN ISO 10272-3 -semi quantitative detection (enumeration) is being developed and due for publication in 2009. This Standard is based on NMKL Method No 119 for drinking water. The first draft of EN ISO 10272-4 Detection of *Campylobacter* in samples from primary production stage - from farm to slaughter (e.g. caeca) will be available before end 2008.

For the validation of EN ISO 10272-1and EN ISO 10272-2 in 2009 various issues need to be considered e.g., Bolton Broth not selective enough, choice of enrichment media depends on matrix, microflora, incubation temperature, time and isolation media. mCCDA would be the first choice for selective medium, while the second should have different principles of selection from mCCDA. CRL will consult with NRL's for their input.

12. Dr Boel Harbom (CRL) gave a presentation on the voluntary PCR ring test organised in September 2008. Its purpose was to assess traditional PCR's undertaken by NRL's. After reviewing publications and PCR's used by NRL's and the CRL, 3 assays were chosen: Linton et al 1997, Denis et al 1999, Wang et al 2002.

25 NRL's participated and 11*Campylobacter* isolates from various sources were used. For the Linton PCR, 16 out of 25 NRL's gave the correct results, for the Denis PCR, 17 out of 25 NRL's gave the correct results and for the Wang PCR 16 out of the 25 NRL's gave the correct results. The *Campylobacter* lari isolate caused the most difficulty to identify.

13. Dr Eva Olsson Engvall (CRL) concluded the workshop by outlining supports available to NRL's from the CRL. These include scientific and technical assistance and laboratory training. In 2009 the CRL will be involved in ISO validation studies, public health contacts and updating the web page. Further ring tests in detection and enumeration of *Campylobacter* in broiler meat will be carried out in 2009 using freeze-dried cultures if possible. An extra ring test may be carried out for molecular typing possibly using MLST. The CRL also plans to extend their database for baseline isolates to include information on molecular typing etc.

Third Annual Workshop of the Community Reference Laboratories for E. coli, , ISS, Rome, 5th December 2008.

NRL Representative: *Dr J. Egan (CVRL).*

29 National Reference Laboratories from 27 EU Member States (MS) attended. The UK and Bulgaria had not yet designated National Reference Labs.

1. Dr. Flemming Scheutz gave an update on the ECDC food borne and water borne disease network (FWD) and surveillance of VTEC infections in Europe. The first meeting of the FWD network took place on the 1st and 2nd October, 2008 in Sweden. The network would initially focus on 20 diseases and aim to progress harmonisation of reporting, disease underreporting and prioritisation of activities. One of the issues in need of consideration was the integration of molecular typing into food, disease and human databases. Core information was required in a real time manner and specific typing methods need to be identified and agreed with stakeholders. There needs to be a standardisation of nomenclature, quality assurance an access for all MS. One priority was PFGE typing for *Salmonella*, *Listeria* and VTEC. Many NRL's were not aware of the FWD and there had been little communication from members to the National Reference Laboratories.

2. Dr. Scheutz outlined the difficulties in comparing the data from year to year due to the variety of different notification and reporting systems in MS. A more harmonised data set would enable better evaluation of the overall situation regarding the importance of VTEC as a zoonotic disease in the EU. Most surveillance on food and animals focus on the O157 serotype making it difficult to assess the potential human health risk from other serotypes in these sources.

Dr Scheutz also gave a brief update on detection and typing of vtx genes. Some standardisation was essential including use of

the same typing methods for the human and animal sectors. More detailed epidemiological studies are necessary to determine the important virulence factors associated with human infection. In addition more detailed methods are needed for the detection of STEC in foodstuffs. Data collected through various programmes indicated that the distribution of VTEC serotypes between countries is not uniform but that this may be strongly influenced by the detection method used and the criteria applied for testing.

3. Dr. Beloeil, EFSA outlined the VTEC situation in Europe as collated from the 2007 Zoonoses Report. 20 MS reported data on human infections in 2007. There were a total of 2,801 cases of VTEC in the EU representing a 43% decrease from 2006. However this decrease may be largely explained by the Czech Republic not reporting any cases in 2007. In 2006 the Czech Republic data accounted for over 31% of total confirmed human cases reported in the EU. The overall notification rate of VTEC infection reported by the 20 Member States was 0.7 cases per 100,000 population. The United Kingdom and Germany accounted for 72% of all cases in the EU in 2007. VTEC continues to be mainly a Northern European problem. In Ireland there were 2.7 cases per 100,000 population compared to 1.9 in the UK and 2.9 in Denmark, Norway and Sweden. Lower cases were reported in Southern Europe.

The majority (76.1%) of reported confirmed human VTEC infections in 2007 were associated with the O157 serogroup. The United Kingdom provided for 66.8% of all serogroup data and UK surveillance focuses mainly on O157. Differences in serotype data in 2007 compared with 2006 are primarily a result of the absence of data from the Czech Republic. The top 5 VTEC serogroups reported in humans in 2007 were O157 (77.8%), O26 , O103, O91 and O121.

A total of 93 cases of haemolytic uraemic syndrome (HUS) were reported in 2007. The majority of HUS cases were reported from Germany, Italy and UK. Most of the reported HUS cases were associated with VTEC O157 with the highest number of cases occurring in young children in the 0-4 age group.

20 MS and 1 non MS reported data on VTEC findings in food and 14 MS reported data in animals for 2007. Data submitted show that 0.5% of approximately 1,000 raw milk samples, 1.1% almost 2,000 cheese samples, 0.3% of 6,461 bovine meat samples and 0.3% of 7,654 mince meat were VTEC positive. The data presented indicate that VTEC prevalence in a variety of foods was generally low and relatively consistent in recent years.

In recent years a number of VTEC O157 outbreaks have been attributed to vegetables. Data submitted for the 2007 report indicate that VTEC O157 is not common in vegetables in European countries.

Approximately 3% of cattle tested were positive for VTEC. Most animals were sampled at slaughterhouses. O157 is the serogroup most frequently reported with only limited data available on other serogroups. Several MS reported limited data on sheep and goats.

4. Dr. Guanghui Wu (VLA, Weybridge) gave a presentation on rapid genotyping of VTEC isolates using a miniaturised microarray chip. Identibac is a range of new bacterial genotyping kits from the VLA based on innovative ArrayTube platform technology from Clondiag Chip Technologies. The system identifies the presence of a target gene or genes in a bacterial isolate when compared to a known gene sequence. The system is simple, rapid and cost effective method that can be used in high throughput or initial screening procedures. IdenitBacEC allows identification of virulence genes associated with a range of E.coli pathotypes. An optimised set of probes is available for detecting 72 gene targets after amplification and the system can process 24 samples simultaneously in a period of 5h obtains high throughput and is relatively cheap.

5. Dr Christine Vernozy (Fr) presented results of a study on the Prevalence of Shiga toxin producing E. coli in frozen mince beef in France. A total of 3,354 mince beef samples were tested by culture and molecular techniques. Only 11 samples were positive on culture for STEC strains; 5 E. coli O157:H7, 3 x O103, 2 x O26 and 1 x O111. However 964 enrichments broths were positive on PCR for stx genes with 172 positive on PCR for stx and eae. While the results of culture demonstrated that the frozen mince was of low potential danger to humans there would be a difficulty created if data was interpreted solely on the basis of molecular analysis.

6. Dr. Gaia Scavia presented the results of the second inter-laboratory study on VTEC identification and typing. 28 NRL's from 22 member states participated; 7 more than in the 2007 ring trials. Strains used in the trial included the top 5 serogroups

(O145, O111, O157, O103, O26) and two others (O121, O91) associated with outbreaks. Results indicated that 62% of all characterisations by NRL's were correct with a further 6% incorrect. 4 NRL's mis-typed O21 as O103 and 1 NRL failed to serogroup O103 and O26. NRL's serogrouped the top 5 isolates correct except for 2 false results. 3 NRL's did not perform the conventional PCR as requested and instead used their in-house RT-PCR methods. 15 of the 28 NRL's detected the vtx2 and eae genes correct and 14 of the 28 also correct for vtx1 gene. Overall the trial results were excellent for the vtx2 genes, satisfactory for the vtx1 gene and excellent for the molecular serotyping.

7. Dr. Beloeil (EFSA) presented an update on the EFSA group working on proposals for a harmonised monitoring of VTEC in animals (especially cattle) and food. One of the most cost effective sampling strategies under discussion for a harmonised monitoring of animals was swabbing hides (brisket area) of cattle and wool of sheep at the abattoir. Testing would be primarily for VTEC O157 but other serogroups (O26, O103, O11 and O145) may also be considered. Cattle between 3 and 24 months of age and sheep between 4 and 12 months of age would be the categories for sampling between April and October 1st each year. The hide and brisket area has been selected as the most contaminated part of the animal. A prevalence of around 12% with a 4% accuracy would lead to approximately 254 samples from both cattle and sheep being required for testing.
Although a VTEC sampling strategy was being considered no corrective actions were being considered in the event of positive results. Dr. Kris De Smet stated that the EU Commission would be meeting with MS early in 2009 to discuss various proposals for harmonising monitoring of pathogens in MS.

Report

Codex Alimentarius AdHoc Intergovernmental Task Force (TFAMR) on Antimicrobial Resistance, Second meeting , Seoul, Republic of Korea, October 2008.

The 2nd meeting of the TFAMR was attended by 132 delegates from 33 Member countries, 1 Member organization and Observers from 7 international organizations. Dr John Egan (DAFF) was the Irish delegate. The full report is available at http://www.codexalimentarius.net/web/archives.jsp?lang=en

At the outset of the meeting the FAO representative drew attention to the joint FAO/WHO Global Initiative for Food-related Scientific Advice on Food Safety (GIFSA) which could support developing countries' efforts to generate and analyse data for risk assessments. The WHO representative informed the task force about WHO activities on containment of foodborne antimicrobial resistance (AMR) and of ongoing discussion between FAO, WHO and OIE on a joint initiative on integrated surveillance of antimicrobial resistance. FAO activities have been strengthened for the last ten years following the alarming increase of antimicrobial resistance in a holistic manner, considering human use, as well as non-human use, of antimicrobials. The task force noted the establishment of the WHO Advisory Group on Integrated Surveillance of Antimicrobial resistance (WHO-AGISAR).

Three TFAMR working groups (WG) had being drafting separate documents on Risk Assessment Guidance, Risk Profiles and Risk Management Guidance to Contain Foodborne Antimicrobial Resistant Microorganisms. The Task force agreed to consolidate the three documents produced by the three working groups (WG's) on into a single document. As it was not feasible, in the time available to appropriately merge the three documents, it was agreed to establish an electronic working group, hosted by the United States of America, open to all Members and observers to prepare the consolidated document taking into account decisions taken by the current session and comments to be received. It requested the electronic working group to prepare revised version by end of May 2009 for circulation for comments at Step 3 and further consideration at its third session. The Delegations of Canada, France and Denmark offered their assistance and cooperation in accomplishing this revision.

WG. DEVELOPMENT OF SCIENCE-BASED RISK ASSESSMENT GUIDANCE REGARDING FOODBORNE ANTIMICROBIAL RESISTANT MICROORGANISMS (AMR-RA)

The scope of this document encompasses the overall risk to human health relating to antimicrobial resistant microorganisms and resistance determinants in microorganisms, in particular in food, food animals, food production/processing, and plants arising from the non-human use of antimicrobials. The scope of the risk assessment will be determined by the risk managers in consultation with risk assessors. Considering the complexity of the AMR issue, specific issues raised or questions asked by risk managers need to be as precise as possible (e.g. combinations of microorganism/antimicrobial class, particular use of antimicrobial, target species, specific geographical area, management of animal and plant production) for risk assessors to specifically address the

risk issue.

Possible sources of data for an AMR-RA include

• Monitoring and surveillance programs including active and passive surveillance (phenotypic and if applicable genotypic information) for AMR derived from humans, food, animal feed, animals, or plants taking into consideration epidemiologic and microbiological breakpoints,
• Epidemiological investigations of outbreaks and endemic cases associated with resistant microorganisms,
• Clinical studies including case reports on the relevant foodborne-related infectious disease prevalence, primary and secondary transmission, antimicrobial therapy, and impacts of resistance on disease frequency and severity,
• Regional treatment guidelines for zoonotic pathogens including information on the medical importance of and potential impacts of increased resistance in target or other microorgansims to alternative treatments,
• Studies on interaction between microorganisms and their environment through the farm-to-table (litter, water, faeces, and sewers),
• Non-human antimicrobial use data such as quantity of antimicrobial drugs used at national and regional levels, daily dosage, species-specific (including plants), route of administration, and duration,
• Investigations of the characteristics of resistant microorganisms and resistance determinants (in-vitro and in-vivo studies),
• Research on properties of antimicrobials including their resistance selection (in-vitro and in-vivo) potential and transfer of genetic elements and the dissemination of resistant microorgansims in the environment,
• Field animal trials addressing the linkage of antimicrobial usage and resistance,
• Information on factors influencing the transfer of resistance determinants,
• Information on the link between resistance, virulence, and/or fitness (e.g. survivability or adaptability) of the microorganism,
• Data on the pharmacokinetics / pharmacodynamics related to the application of drugs.

The process of an AMR-RA is composed of Hazard Identification, Exposure Assessment, HazardCharacterization, and Risk Characterization.

The hazard identification process recognizes that the hazards, resistant pathogenic and commensal microorganisms and/or resistance determinants of food, animal feed, and/or of animal/plant origin, have the potential to cause an adverse human health effect. Data in the hazard identification step may include: description of the microorganisms and their genotypic and phenotypic characteristics including molecular characterization of resistance determinants, virulence and pathogenicity, in-vivo studies in laboratory animals, surveillance or epidemiological studies of resistant infections or resistance determinants, and clinical studies. Additionally, interaction of resistant microorganisms or resistance determinants with the environment (e.g., interactions in animal feeds or aquaculture environment as well as in food matrices), and information on the susceptible strains of the same organisms or related resistant microorganisms (or resistance determinants) will be useful.

The exposure assessment will address all the pathways as a consequence of non-human uses of antimicrobials resulting in the emergence and dissemination of resistant microorganisms and resistance determinants to humans via the food chain. The fundamental preliminary activities in this step should therefore include: (a) clear depiction or drawing of the exposure pathway; (b) detailing the necessary data requirements based on this pathway; and (c) summarizing the data.
Listed below are possible factors under consideration for an exposure assessment that influence the development, prevalence and transmission of resistance microorganism and resistance determinants for which pre-harvest and post harvest data may be required

(1) Selection Pressure

Extent of antimicrobial agent use or proposed use
• Number of animal, crop or target farms exposed to the antimicrobial agent in the defined time period,
• Geographical distribution of use and/or farms,
• Extra- and off-label use of antimicrobial agent,
• Data on trends in antimicrobial use and information on emerging diseases, changes in farm production system, or other changes that are likely to impact antimicrobial use.

(2) Intensity of non-human use of antimicrobials

• How much is used per target (as quantitative as possible) in the defined time period,
• Methods and routes of administration of the antimicrobial

agent
(individual / mass medication, local/systemic application),
• Dosing regimen and duration of use,
• Number of administrations / administration periods in the defined time period,
• Cumulative effects of use of other antimicrobials in the defined time period.

(3) Target animal or crop and microbial factors affecting resistance development and spread

• Seasonal changes in microorganism prevalence,
• Rate of resistance development in commensal and zoonotic microorganisms in targets after administration of an antimicrobial agent,
• Resistance mechanisms, location of resistance determinants, occurrence and rate of transfer of resistance between microorganisms,
• Cross-resistance and/or co-selection for resistance to other antimicrobials (phenotypic or genotypic description),
• Prevalence of commensals and zoonotic microorganisms in targets and proportion resistant to the antimicrobial (and minimal inhibitory concentration levels),
• Primary and secondary transmission among targets,
• Animal management factors affecting immunity,
• Plant management.

(4) Initial level of contamination of the food product

• Antimicrobial resistance rate of microorganisms present in/on the target at slaughter or time of crop harvest,
• Antimicrobial resistance rate of microorganisms present in the retail food,
• Food matrix factors (food product formulation).

(5) Food production factors

• Sanitation and process controls, such as GMP, GHP and HACCP,
• Methods of production and processing,
• Points for cross-contamination,
• Packaging,
• Probable use of additives and preservatives (due to their activities or impacts on growth or numbers of microorganisms),
• Starter cultures (type and number of microorganisms) used as ingredients
• Distribution, and storage,
• Regional or seasonal differences in quantity of food products produced,
• Catering and food services.

(6) Consumer behaviours

• Storage, cooking and handling,
• Cross-contamination,
• Human-to-human transmission of the microorganisms, including personal hygiene,
• Overall per capita consumption,
• Patterns of consumption and socio-economic, cultural, ethnic and regional differences.

(7) Microbial factors

• Capacity of food-derived resistant microorganisms to transfer resistance to human commensal and/or pathogenic microorganisms,
• Growth and survival characteristics of resistant microorganisms.

The hazard characterization step considers the characteristics of the pathogen as already described in the hazard identification step, matrix and host in order to determine the probability of disease upon exposure to the pathogen. The hazard characterization step estimates the probability of infection, and then conditional to this event, estimates the probability of disease.

Possible data requirements for hazard characterization include;

(i) Resistant microorganisms and resistance determinants

• Resistance genotype and phenotype including cross-resistance and co-resistance,
• Transferability (mobile elements) and persistence,
• Pathogenicity, virulence and their linkage to resistance,
• Food matrix related factors that can influence the survival capacity of the microorganisms while passing through the gastro-intestinal tract.

(ii) Adverse health effect characteristics
• Nature of the infection/disease,
• Host factors and susceptible population,
• Diagnostic aspects,
• Treatment with antimicrobial agent and hospitalization,

• Severity of adverse health effects,

• Epidemiological pattern (outbreak or endemic),

• Persistence of hazards in humans.

The risk characterization step of AMR-RA integrates the information from the preceding components of the risk assessment and synthesizes overall conclusions about risk that is complete, informative and useful for risk managers. The purpose of risk characterization is to answer the original questions posed by risk managers and to put into context the findings from the risk assessment process including uncertainties and other findings that could have an impact on the risk management decision possible factors for modelling the likelihood for the development and spread of resistance within animal or plant populations.

Potential Points for Consideration in the Risk Characterization include;

(i) Factors in risk estimation include

• Number of people falling ill and the proportion of that number with resistant strains of microorganisms,

• Increased severity or duration of infectious disease due to resistance,

• Number of person-days of disease per year,

• Deaths (total per year; probability per year or lifetime for a random member of the population or a member of a specific more exposed or more vulnerable subgroup

• Importance of pathology caused by the target microorganisms,

• Absence of alternative antimicrobial agent and alternatives with potential toxicity,

• Alternatives available in case of resistance, and potential impact of switching to alternative antimicrobial agent,

• Incidence of resistance,

• Consequences to allow weighted summation of (e.g. disease and hospitalization) or some arbitrary scale of impact to allow weighted summation of different risk impacts.

(ii) Scientific evaluation of risk management options include

• Comparison of public health burden before and after interventions.

WG. DEVELOPMENT OF GUIDANCE ON CREATING RISK PROFILES FOR ANTIMICROBIAL RESISTANT FOODBORNE MICROORGANISMS FOR SETTING RISK ASSESSMENT AND MANAGEMENT PRIORITIES

In the context of this document, a potential food safety issue may arise when antimicrobial resistant microorganisms and antimicrobial resistance determinants are present in food and feed, including aquaculture, or are transmitted through food and animal feed. Foodborne exposures to resistant microorganisms or resistance determinants may adversely impact human health. The risk manager initiates the risk management process to evaluate scope and magnitude of the food safety issue and, where necessary, to commence activities to manage the identified risk.

AMR food safety issues may be identified on the basis of information arising from a variety of sources, such as antimicrobial resistance surveillance in animals and in foods of animal origin, food safety monitoring, antimicrobial usage surveys, animal and human surveillance data (including post- marketing surveillance data on approved antimicrobials), epidemiological or clinical studies, laboratory studies, research on resistance transfer, scientific, technological or medical advances, environmental monitoring, recommendations of experts and interested parties, etc. Information on antimicrobial resistance microorganisms and resistance determinants related to plant production and food processing should be included.

The AMR risk profile describes a food safety problem and its context that presents, in a concise form, the current state of knowledge related to the food safety issue, describes current control measures and risk management options that have been identified to date, if any, and the food safety policy context that will influence further possible actions. The risk profile is usually developed by personnel with specific scientific expertise on the food safety issue of concern and some understanding of antimicrobial resistance risk assessment techniques. Interested parties who are familiar with the relevant production chain and related production techniques should be consulted.

The relevance of lists of critically important antibiotics developed by "national" groups in risk profiling was discussed with most delegations indicating that lists developed by national groups provided useful elements in the preparation of risk profiles. The TFAMR, noting that as the issue of "provisional decision" was discussed by both the RP and RM WG's, agreed to postpone further discussions on the issue to a later date.

Given the potentially high resource costs associated with con-

ducting risk assessments and/or implementing risk management goals, a risk ranking or prioritization process is important in placing the risks from a specific food commodity + antimicrobial resistant microorganism + antimicrobial use combination in context with other risk scenarios that require the attention of risk managers. The output from the risk profile provides the principal criteria that should be used by risk managers in this risk ranking or prioritization process.

Beyond the description of the food safety issue provided by the risk profile, other criteria may be used for ranking or prioritization; these are generally determined by the risk mangers in conjunction with stakeholders, and in consultation with risk assessors on scientific aspects of the issues. Such criteria may include;

- Perceived relative level of risk to consumers,
- Capability to implement effective food safety control measures,
- Potential international trade implications associated with food safety control measures,
- Regulatory challenges,
- Policy concerns / public demand.

Following development of the risk profile and the conduct of the risk ranking/prioritization steps, risk managers should decide on the preliminary risk management goals that determine the next steps to be taken, if any, to address the identified food safety issue. The TFAMR had extensive discussions on broad risk management goals and whether they should be part of RP or RM activities. Goals should be established through an interactive process between the risk managers, scientific experts, and other interested parties and have as their primary objective the protection of the health of consumers. Other considerations in selecting appropriate risk management goals include the potential impact on trade, as well as the feasibility of implementation, enforcement, and compliance of the risk mitigation measures associated with the goals.

Often critical in establishing and achieving risk management goals is determining the need, or the feasibility, of a risk assessment. Factors that may increase the desirability of a risk assessment include;

- The nature and magnitude of the risk are not well characterized,
- The risk is connected to economic, social, and cultural considerations, including consequences for animal health and welfare,
- The risk management activities have major trade implications.

Other practical issues that impact the decision as to whether a risk assessment is needed include;

- The availability of resources,
- The urgency of the food safety issue,
- The availability of scientific information.

Suggested elements to include in an AMR risk profile are listed below.

A. Definition of the hazard-food commodity combination(s) of concern include;

- Food commodity;
- Antimicrobial resistant pathogen,
- Antimicrobial use pattern,
- Description of the food commodity and the associated cause for concern (e.g., antimicrobial resistant foodborne disease, trade restrictions) due to the hazard,
- Occurrence of the hazard in the food chain.

B. Description of the public health problem (i.e., the adverse human health consequences) include;

(i) Characteristics of the resistant microorganism(s) or resistance determinants, including key attributes that are the focus of its public health impact (e.g., cross resistance, co-resistance, horizontal gene transfer);

- Growth rate

(ii) Characteristics of the antimicrobial-susceptible infection, disease, including;

- Susceptible populations;
- Annual incidence rate in humans including, if possible, any differences between age and sex;
- Severity of clinical manifestations (e.g., case-fatality rate, rate of hospitalization;
- Nature and frequency of long-term complications.

(iii) Characteristics of the antimicrobial-resistant infection, disease;

- Added burden of the infection, disease due to antimicrobial resistance, if readily available (e.g., medical and/or hospital costs; working days lost due to disease, etc.),
- Evidence of links between resistance, virulence, and/or fitness of the antimicrobial resistant microorganism.

(iv) Characteristics of treatment of the antimicrobial resistant infection, disease;

- Options for treating the infection, disease (e.g., importance of antimicrobial drug for treatment of human adverse health effect, possible side effects of alternate treatments),
- Extent of human use of the antimicrobial agent for which resistance is the concern;
- Availability and nature of treatment,
- Prevalence of resistance in human populations.

C. Description of food commodities associated with the antimicrobial resistant microorganisms or resistance determinants (Post-harvest factors) include;

- Characteristics of the food commodity (commodities),
- Food use and handling that influences transmission of the hazard,
- Frequency and characteristics of foodborne sporadic cases, Epidemiological data from outbreak investigations,
- Prevalence of resistance on food commodity,
- Evidence of a relationship between the presence of the antimicrobial resistant microorganisms or resistance determinants on the food commodity and the occurrence of the adverse health effect in humans.

D. Description of antimicrobial(s) include;

- Chemical, physical and pharmacological properties of the antimicrobial agent,
- Type of use (treatment/ prevention/control/ growth promotion),
- Dose regimen and route of administration,
- Final product specifications,
- Specific rules of usage for the country concerned,
- Quantity of use in relevant animal and plant species,
- Factors influencing the persistence of resistance in the pre-harvest production stage,
- Associations between usages and development and persistence of resistance,
- Factors that may affect the dissemination of antimicrobial resistant microorganisms through the food chain,
- Evidence of a relationship between the use of the antimicrobial and the occurrence of antimicrobial resistant microorganisms, or resistance determinants, in the food commodity of concern,
- Persistence of the antimicrobial in the environment, and factors affecting the maintenance of antimicrobial resistant microorganisms and/or resistance determinants,
- Contribution of alternative (non-foodborne) sources of antimicrobial resistance.

E. Antimicrobial resistance genes and resistance determinants include;

- Factors that may affect the frequency of transfer of genetic elements through the food chain,
- Description of the molecular genetics of the antimicrobial resistance of concern.

F. Other Risk Profile Elements include,

- Summary of the extent and effectiveness of current risk management practices including food safety production/ processing control measures, educational programs, and public health intervention programs (e.g., vaccines),
- Identification of additional risk mitigation strategies that could be used to control the hazard,
- The extent of international trade of the food commodity,
- Existence of regional/international trade agreements and how they may affect public health with respect to the specific hazard-food commodity combination(s),
- Public perceptions of the problem and the risk,
- Initial assessment of the need and benefits to be gained from requesting an antimicrobial resistance risk assessment, and the feasibility that such an assessment could be accomplished within the required time frame,
- Importance of antimicrobial drug to animal medicine,
- Availability of alternative treatments and preventive measures.

G. Assessment of available information and major knowledge gaps include;

- Existing antimicrobial resistance risk assessments on the food commodity + antimicrobial resistant pathogen + antimicrobial use combination(s) including, if possible,
- Other relevant scientific knowledge and data that would facilitate risk management activities including, if warranted, the conduct of a risk assessment,
- Existing Codex guidance documents,
- International and/or national governmental and/or industry codes of hygienic practice and related information,
- Areas where major absences of information exist that could hamper risk management activities, including, if warranted, the conduct of a risk assessment

WG. DEVELOPMENT OF RISK MANAGEMENT GUIDANCE TO CONTAIN FOODBORNE ANTIMICROBIAL RESISTANT MICROORGANISMS

The main purpose of the RM guideline was to reduce the risk of foodborne AMR microorganisms and resistance determinants from the non-human use of antimicrobials following risk profiling and/or risk assessment.

Risk management options should consider the relevant practice throughout the food chain and could be divided in pre-harvest and post-harvest aspects. Pre-harvest options would contain aspects such as Responsible Use Guidelines and Codes of Practice for antimicrobial agents and possibilities to modify the use of antimicrobial agents as related to the risk of the development of antimicrobial resistance microorganisms used in food production. Post-harvest options would contain aspects contributing to minimising the contamination of food by resistant microorganisms such as food hygiene practices for handling and avoiding cross contamination.

In food animal production additional risk management options pre- and post- licencing approval could include regulatory controls on conditions of use, such as marketing status limitation, extra-/off-label prohibition, extent of use limitation. The level of control could be implemented in a stepwise fashion proportionate to the risk with consideration of critically important antimicrobials or as needed to obtain a consumer protection or food safety goal.

Whenever possible, a microbial diagnosis and susceptibility testing should be performed prior to treatment for a given infection. National authorities may support the development and dissemination of standards for establishing culture and susceptibility testing, breakpoints, and interpretive criteria determinations for important pathogens and antimicrobials approved for use in food animals. Additional options may include recommendations on different AM to be used, if several antimicrobials can be used for a given indication in an animal, development of prudent use guidelines that are species- and disease condition-specific by professional bodies, avoiding disease prevention/prophylactic use in healthy animals not considered to be at risk of infection or prior to the onset of clinical infectious disease and minimizing the presence and transmission of foodborne microorganisms and determinants between animals, from animals to humans and between flocks/holdings by implementing animal health and infection control programs so as to reduce the risk associated with the use of antimicrobials.

In food crop production additional risk management options in the pre- and post-approval and licensing of antimicrobials could include regulatory controls on conditions of use, such as marketing status limitation, extent of use limitation. The level of control could be implemented in a stepwise fashion proportionate to the risk with consideration of Critically Important Antimicrobials. Competent authorities and/or professional bodies should elaborate crop species-specific prudent use treatment guidelines in consultation with all relevant interested parties. Prudent use guidelines should contain information such as use of culture and susceptibility, breakpoints, and interpretive criteria. National authorities may also support the development and dissemination of standards for establishing culture and susceptibility, breakpoints, and interpretive criteria for important pathogens and antimicrobials approved for use in crops.

Additional measures could also include dischouraging the use of antimicrobials on healthy crops preventing the presence and transmission of foodborne resistant microorganisms and resistance determinants between crops and from crops to humans by implementing biosecurity and infection control programs.

Due to time constraints it was not feasible to progress many other aspects of the RM guideline. The TFAMR recognised the need for further work on risk management options to clarify its scope and to include possible advantages and disadvantages of the most important management options and examples on experiences of voluntary application of guidelines on prudent use versus restrictive actions.

Matrix/ Parameter	CRL	Head of NRL	Contact person (if different to Head NRL)
Milk and milk products	AFSSA - Laboratoire d'études et de recherches sur la qualité des aliments et sur les procédés agroalimentaires (LERQAP) F-94700 Maisons-Alfort, France	Bernadette Hickey Tel: +353 1 6157452 Fax: +353 1 6157454 Email: Bernadette.hickey@agriculture.gov.ie	
Zoonoses (salmonella)	Rijksinstituut voor Volksgezondheid en Milieu (RIVM) 3720 BA Bilthoven The Netherlands	Dr John Ferris Tel: +353 1 6157106 Fax: +353 1 6157197 Email: john.ferris@agriculture.gov.ie	Dr Montserrat Gutierrez Tel: +353 1 6157222 Fax: +353 1 6157116 Email: mm.gutierrez@agriculture.gov.ie
Listeria monocytogenes	AFSSA - Laboratoire d'études et de recherches sur la qualité des aliments et sur les procédés agroalimentaires (LERQAP) F-94700 Maisons-Alfort France	Bernadette Hickey Tel: +353 1 6157452 Fax: +353 1 6157454 Email: Bernadette.hickey@agriculture.gov.ie	
Coagulase positive *Staphylococci*, including *Staphylococccus aureus*	AFSSA - Laboratoire d'études et de recherches sur la qualité des aliments et sur les procédés agroalimentaires (LERQAP) F-94700 Maisons-Alfort France	Bernadette Hickey Tel: +353 1 6157452 Fax: +353 1 6157454 Email: Bernadette.hickey@agriculture.gov.ie	
Escherichia coli, including Verotoxigenic E. Coli (VTEC)	Istituto Superiore di Sanità (ISS) I-00161 Roma Italy	Dr John Ferris Tel: +353 1 6157106 Fax: +353 1 6157197 Email: john.ferris@agriculture.gov.ie	Dr Montserrat Gutierrez Tel: +353 1 6157222 Fax: +353 1 6157116 Email: mm.gutierrez@agriculture.gov.ie
Campylobacter	Statens Veterinärmedicinska Anstalt (SVA) S-751 89 Uppsala Sweden	Dr John Ferris Tel: +353 1 6157106 Fax: +353 1 6157197 Email: john.ferris@agriculture.gov.ie	Dr John Egan Tel: +353 1 6157138 Fax: +353 1 6157116 Email: john.egan@agriculture.gov.ie
Parasites (in particular *Trichinella*, *Echinococcus* and *Anisakis*)	Istituto Superiore di Sanità (ISS) I-00161 Roma Italy	Dr John Ferris Tel: +353 1 6157106 Fax: +353 1 6157197 Email: john.ferris@agriculture.gov.ie	Paul Rafter/Dr Tom Murphy Tel: +353 1 6157350 Fax: +353 1 6157361 Email: paul.rafter@agriculture.gov.ie
Antimicrobial resistance	Danmarks Fødevareinstituttet DK-1790 København V Denmark	Dr John Ferris Tel: +353 1 6157106 Fax: +353 1 6157197 Email: john.ferris@agriculture.gov.ie	Dr Montserrat Gutierrez Tel: +353 1 6157222 Fax: +353 1 6157116 Email: mm.gutierrez@agriculture.gov.ie
Animal proteins in feedingstuffs	Centre Wallon de recherches agronomiques (CRA-W) B-5030 Gembloux, Belgium	Gabriel Roe Tel: +353 1 6302902 Fax: +353 1 6280634 Email: Gabriel.roe@agriculture.gov.ie	
Transmissible spongiform encephalopathies (TSEs)	The Veterinary Laboratories Agency Surrey KT15 3NB United Kingdom	Dr John Ferris Tel: +353 1 6157106 Fax: +353 1 6157197 Email: john.ferris@agriculture.gov.ie	Dr Paul Collery Tel: +353 1 6157203 Fax: +353 1 6157199 Email: paul.collery@agriculture.gov.ie
Chemical elements in food of animal origin	Istituto Superiore di Sanita Viale Regina Elena 299 00161 Rome, Italy.	Dr John Ferris Tel: +353 1 6157106 Fax: +353 1 6157197 Email: john.ferris@agriculture.gov.ie	Paul Rafter Tel: +353 1 6757350 Fax: +353 1 6157361 Email: paul.rafter@agriculture.gov.ie

Matrix/ Parameter	CRL	Head of NRL	Contact person (if different to Head NRL)
Residues in food of animal origin *Beta-agonists* *Anthelmintics* *Anticoccidials including* *Nitroimidazoles* *Non-steroidal anti-inflammatory drugs (NSAID´s)*	Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL) D-10562 Berlin GERMANY	(1) Dr John Ferris Tel: +353 1 6157101 Fax: +353 1 6157197 Email: john.ferris@agriculture.gov.ie (2) Michael O'Keeffe Tel: +353 1 8059500 Fax: +353 1 8059550 Email: michael.okeeffe@teagasc.ie (3) Dermot Hayes Tel: +353 1 505 7000 Fax: +353 1 505 7070 Email: dermot.hayes@statelab.ie	(1) Paul Rafter Tel: +353 1 6757350 Fax: +353 1 6157361 Email: paul.rafter@agriculture.gov.ie (2) Liam Regan Tel: +353 1 5057062 Fax: +353 1 505 7070 Email: liam.regan@statelab.ie
Residues of animal origin *Stilbenes, stilbene derivatives, salts and esters* *Antithyroid agents* *Steroids* *Resorcylic Acid Lactones (RAL´s) incl. zeranol* *Sedatives* *Mycotoxins*	Rijksinstituut voor Volksgezondheid en Milieu (RIVM) 3720 BA Bilthoven The Netherlands	(1) Dr John Ferris Tel: +353 1 6157106 Fax: +353 1 6157197 Email: john.ferris@agriculture.gov.ie (2) Dermot Hayes Tel: +353 1 505 7000 Fax: +353 1 505 7070 Email: dermot.hayes@statelab.ie	(1) Paul Rafter Tel: +353 1 6757350 Fax: +353 1 6157361 Email: paul.rafter@agriculture.gov.ie (2) Liam Regan Tel: +353 1 5057062 Fax: +353 1 505 7070 Email: liam.regan@statelab.ie
Residues of animal origin Antibacterial substances, including *sulphonamides* and *quinolones* *Dyes* *Carbadox and olaquindox* *Chloramphenicol* *Dapsone* *Nitrofuranes*	Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants AFSSA-. F-35302 Fougères France	(1) Dr John Ferris Tel: +353 1 6157106 Fax: +353 1 6157197 Email: john.ferris@agriculture.gov.ie (2) Michael O'Keeffe Tel: +353 1 8059500 Fax: +353 1 8059550 Email: michael.okeeffe@teagasc.ie	Paul Rafter Tel: +353 1 6157350 Fax: +353 1 6157361 Email: paul.rafter@agriculture.gov.ie
Residues of pesticides in cereals	Danish Institute for Food and Veterinary Research (DFVF) DK 2860 SØborg Denmark	Dan O'Sullivan Tel: + 353 1 6157610 Fax: + 353 1 6157575 Email: dan.osullivan@agriculture gov.ie	
Residues of pesticides in food of animal origin	Chemisches und Veterinäruntersuchungsamt (CVUA) Postfach 100462 D-79123 Freiburg Germany	Dan O'Sullivan Tel: + 353 1 6157610 Fax: + 353 1 6157575 Email: dan.osullivan@agriculture gov.ie	
Residues of pesticides in fruits and vegetables	Pesticide Residue Research group Universidad de Almería (PRRG) Laboratorio Agrario de la generalitat valenciana (LAGV) Spain	Dan O'Sullivan Tel: + 353 1 6157610 Fax: + 353 1 6157575 Email: dan.osullivan@agriculture gov.ie	
Pesticides:single residue methods	Chemisches und Veterinäruntersuchungsamt (CVUA) Stuttgart Postfach 1206 D-70702 Felbach Germany	Dan O'Sullivan Tel: + 353 1 6157610 Fax: + 353 1 6157575 Email: dan.osullivan@agriculture gov.ie	