



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**
Dr C Mannion

Pesticide Residues
Mr M Hickey

Campylobacter
Dr J Egan

Animal Proteins
Dr J Choiseul

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.

In this issue:

- (a) CVRL awarded VetNet certification in Pulsed Field Gel Electrophoresis (PFGE) for *E. coli*.
- (b) Annual Workshop of the Community Reference Laboratory, AFSSA - Workshop on Biological Screening Methods, Fougères, France, 16th - 17th March.
- (c) Forth Annual Workshop of the Community Reference Laboratory on Antimicrobial Resistance, National Food Institute, Berlin, Germany, 8th - 9th April.
- (d) Report: First Meeting of the EU Working Group in Antimicrobial Resistance.
- (e) Fourth Annual Workshop of the Community Reference Laboratory for Animal Proteins (CRL-AP), Turin.
- (f) Fifth Annual Workshop of the Community Reference Laboratory for Parasites Rome, 3rd - 4th June.

CVRL awarded VetNet certification in Pulsed Field Gel Electrophoresis (PFGE) for *E. coli*

Molecular typing of foodborne pathogens is critical for tracing both their sources and distribution along the food chain continuum. In Ireland, the CVRL, as the National Reference Laboratory for some of the major foodborne pathogens such as *Salmonella*, *Campylobacter* and *E. coli*, has been working with other national and international stakeholders to introduce and apply various molecular typing systems across a range of pathogens. In association with UCD, and with funding through the Food Institutional Research Measure, staff from the CVRL were trained in 2009 at the USDA, Richard B Russell Agricultural Research Centre, in PulseNet, standardised PFGE protocols and VetNet BioNumerics software in concordance with the CDC-PulseNet programme.

Following implementation of procedures in the CVRL, the laboratory participated successfully in a number of ring trials and became one of the first non-US laboratories to be awarded VetNet certification in Pulsed Field Gel Electrophoresis. VetNet certification for *Salmonella* was awarded in 2009 and this has now been followed by VetNet certification for *E. coli*.

A range of bacterial isolates from across the food sector in Ireland are now routinely typed using molecular techniques and compared with other isolates from various sources and across many countries where standardised typing techniques are in operation. These techniques have already been applied by the NRL to trace the sources of *Salmonella* in pig meat in Ireland. Such initiatives not only ensure that technologies developed under the various R&D programmes are applied in front line food safety surveillance but also further enhance the existing food safety controls. Efforts are continuing to develop and apply other typing techniques in front line surveillance of food borne pathogens.

Report

NRL Residues of Animal Origin

Annual Workshop of the Community Reference Laboratory, AFSSA - Workshop on Biological Screening Methods, Fougeres, France, 16th - 17th March.

NRL Representative: Dr. Celine Mannion, CMCL

Introduction

The main aim of this workshop was to discuss the validation of biological screening methods and review methods available in minor matrices. Topics discussed included results of proficiency test results, results of the CRL 2009 questionnaire and microbiological methods used for National Residue Control Plans (NRCP's). Current developments in Community residue control legislation were also outlined. The need to develop a protocol of validation for screening test methods was acknowledged and the CRL guideline document on the validation of screening methods with regard to "cut-off" and CCB values was presented in detail.

Eric Verdon (AFSSA) highlighted that CRL's will now be known as European Reference Laboratories (EU-RL's). In France, AFSSA (food safety) will combine with AFSSET (environment and workers) and be renamed ANSES (national agency for safety and security of food, environment and workers).

Roberto Manos (DG SANCO) gave a general overview of developments in Community residue control legislation. He highlighted the replacement of Regulation 2377/90 with 470/2009, which came into force 7th July 2009 and lays down procedures for the establishment of residue limits. In addition Regulation 37/2010 entered into force on 9th February 2010 and replaces previous 4 annexes of Regulation 2377/90. He recommended reviewing CRL's guidelines on validation of screening methods for residues of veterinary medicine which can also be viewed on DG SANCO website.

Further amendments of Directive 96/23/EC are under consideration (the revision of 96/23 should take into account veterinary practices in each member state) and it is planned to integrate residue control into the framework of regulation 882/2004. It was also noted that all current framework contracts are only in place until end 2011.

Mariel Pikkemaat (RIKILT) reviewed the proficiency test on antibiotics in bovine muscle provided in 2009 and queried whether or not antibiotic screening approaches are sufficiently adequate. RIKILT is accredited for the organisation of PTs. Overall the false negative rate of this PT was poor at 53% (73% for microbiological, 50% for biochemical and 22% for instrumental analyses) and confirmatory results were insufficient as laboratories performed confirmatory analyses based on initial screening results only. The overall poor detection of macrolides and aminoglycosides was not considered important by some participants, as the relevant compounds were not widely used in their countries. However this view was not shared or accepted.

Régine Fuselier (AFSSA) discussed recent PTs organised by AFSSA for screening methods, in particular antibiotic screening in milk and Valerie Gaudin (AFSSA) discussed the recent questionnaire and data collected. All NRLs are screening for

antibiotics in milk and are using many different strategies and multiple methods (B-lactams and Tetracyclines). In 2009 PT in milk 26 participants used 63 screening methods. From the 2009 questionnaire it appears that 23, 15 and 13 NRLs are using microbiological, physicochemical and immunological methods for screening milk, respectively. Overall false positive rates are satisfactory but false negative rates are high. It was noted that multiple methods implemented by laboratories screening milk for antibiotics are detecting B-lactams at or below the MRL but not Cefquinome at 2.5 time MRL (including delvo and plate test). The detection of Tetracyclines in milk has improved greatly due to the implementation of specific rapid tests (81% of participants detected OTC at MRL). In contrast, for antibiotic screening in muscle, laboratories are more likely to implement one screening method, usually a plate test. On conclusion it was agreed that laboratories need to choose the best-fitting screening tests for antibiotics in milk. A multi antibiotic residue PT in pig muscle is planned Oct-Nov 2010.

Eric Verdon and Valerie Gaudin presented the 2009 NRL questionnaire on antibiotics and veterinary practices in Europe. NRLs concluded that B-lactams and Tetracyclines were most commonly used followed by Sulphonamides and Macrolides, however, NRLs mostly detected Tetracyclines followed by B-lactams, Sulphonamides and Macrolides. Some countries collect data on antibiotic usage e.g. in Switzerland Sulphonamides are most commonly detected but B-lactams are most commonly prescribed; in UK tetracyclines are most commonly used. Relative importance of antibiotic classes depends on species (BLs and TTC^os in poultry, BLs and Aminoglycosides in bovine); route of administration (intra-mammary vs. oral); age of animals etc. As it is difficult to conclude a common pattern of antibiotic usage for all EU countries it is therefore difficult to know which residues to look for at a glance. In France TTCs, BLs and Sulfas are commonly used but Quinolones, 3rd & 4th generation Cephalosporins and Polypeptides are on the increase.

Valerie Gaudin presented the NRCP 2009 questionnaire on screening methods for muscle and kidney. She highlighted the number of NRLs testing both muscle and kidney for antibiotic residues and different strategies implemented. In total 17 and 16 of the 28 NRLs test both kidney and muscle for antibiotic residues in their NCP and urgent slaughters, respectively. 14 NRLs perform post screening on both matrices and 15 NRLs confirm both matrices. The most commonly used screening methods are plate tests and some NRLs use multiple methods. Many NRLs post-screen positive samples using plate tests, penicillinase etc.

Michel Laurentie (AFSSA) discussed the link between kidney and muscle concentrations and highlighted that levels are higher in kidney than in muscle although rates of depletion are often similar but depend on many factors and should be established for each compound! He concluded that determination in kidney alone is not sufficient and that

confirmation should be carried out in muscle. Antibiotic screening methods should be validated in both muscle and kidney!

Valerie Gaudin presented the CRL guidance document on the validation of screening methods, which was issued to all NRLs in January 2010. It was highlighted that if a screening method is not able to detect all essential target analytes at the regulatory limit in all matrices and species then additional tests must be added. The screening target concentration at which a sample is categorised as "screen positive" must be at or below the regulatory limit (MRL), MRPL, Recommended Concentration or Reference point for action. When choosing analytes to validate analytes within a group, one must use the analyte from each group with the lowest inhibition or least sensitivity. Validation should be carried out using spiked tissue or spiked discs with tissue placed on top. Validation of plate tests using spiked kidney or discs + kidney caused widespread discussion amongst the group as kidney is a very difficult matrix to work with and contains many waste products/inhibitors. Methods should be continually verified by participation in relevant proficiency tests.

Valerie Gaudin gave an overview of the progress of the validation of screening methods in NRLs and concluded that the majority of microbiological screening methods are validated with approx. half of laboratories using spiked matrix and covering antibiotics representative of the group. She outlined the representative antibiotics which may be appropriate for validating a microbiological screening method. It was recommended that at least 1 or 2 representative analytes per family be validated - preferably those essential and least sensitive. However, some participants stated that this resulted in laboratories having to validate all compounds within a family.

AFSSA introduced and outlined a concept of validating a LC-MSMS method for the screening of 58 antimicrobials in milk qualitatively using the current validation of screening methods guideline and the requirements of decision 2002/657/EC. They determined the analytical response at the level of interest for each antibiotic and then turned this into a qualitative result by setting a cut-off value which then classified the samples as "to be confirmed or not". This concept of validation was applicable and achieved its objectives. They also showed how applicable the guideline validation is to all kinds of immunological tests e.g. ELISA, biosensors etc.

Valerie Gaudin discussed the validation of agar diffusion tests for screening antibiotic residues e.g. CCB, selectivity, applicability, ruggedness. The "cut-off" level / diameter of inhibition zone for milk and muscle was 11mm and 2mm, respectively. Samples were spiked at the MRL or levels from pre-validation work, e.g. for Chlortetracycline in milk the CCB (zone diameter > 11mm) was 300ppb or 3xMRL. It was concluded that the process of validation involves a significant workload in terms of operator, time, materials, reagents and matrices.

The Spanish NRL presented validation of the 5-plate test. In Spain, 50 laboratories screen for antibiotic residues. The method used is the Bogaerts & Wolf 4-plate test and an *E. coli* plate. Muscle samples are heated and placed in a hole in the agar. Milk is also placed in a hole in the agar. This method does not detect Sulphonamides and prohibited substances well. They concluded that kidney is preferable to muscle using the 5-plate test and that validating using intact tissue + disc is preferable to minced tissue + disc - CCB less with intact tissue + disc.

Finally AFSSA highlighted the requirements when transferring a microbiological method from one laboratory to another - adherence to protocol, identical reagents, training of personnel, use of positive controls, abridged validation, participation in PTs etc.

Report

NRL Antimicrobial Resistance

Forth Annual Workshop of the Community Reference Laboratory on Antimicrobial Resistance, Berlin, Germany, 8th - 9th April.

NRL Representative: *Dr Montserrat Gutierrez, CVRL*

Proficiency testing:

There were six proficiency tests organised by the EURL-AMR in 2009, five on antimicrobial susceptibility involving the regular strains (*E. coli*, *Enterococci*, *Staphylococcus aureus*, *Salmonella* and *Campylobacter*) and a first on the isolation of MRSA from dust samples.

As in previous years, the antimicrobial susceptibility proficiency tests consisted of categorising strains (eight per pathogen) as either resistant or susceptible for a number of antimicrobial substances. In addition, some strains required further testing to determine resistance mechanisms that are relevant to the particular bacteria, e.g. ESBL in *Salmonella* and *E. coli* and *mecA* gene determination for relevant *Staphylococci*. A maximum of 5% of deviating results for each pathogen was set as the acceptance criteria when assessing performance. The EURL-AMR provided the cut-off points (in mg/l) to be applied for the categorisation of the strains for laboratories using dilution methods. Information regarding species of the strain was also provided if relevant for the interpretation of results, e.g. *C. jejuni* vs. *C. coli* or *E. faecium* vs. *E. faecalis*.

The following table shows a summary of the results:

	No. laboratories participating (No. using MIC)	% Correct results	No. laboratories underperforming
<i>E. coli</i>	28 (6)	98.5%	2
<i>Enterococci</i>	23 (5)	95.8%	8
<i>Staphylococci</i>	27 (11)	98.2%	2
<i>Salmonella</i>	31 (5)	98.4%	2
<i>Campylobacter</i>	26 (0)	97.8%	3

Results of the PT showed that the performance of NRL's has improved from previous years. The main findings were as follows:

- For both *E. coli* and *Salmonella* deviations in results were mainly found in laboratories performing disk diffusion and most failures were related to ciprofloxacin (low level resistance) and cephalosporin resistance.
- For *Enterococci*, deviations were mainly associated with disk diffusion, especially failure to detect high-level resistance to aminoglycosides. A revision of the antimicrobials for testing in future PT's was recommended and in particular omission of avilamycin, daptomycin and tigecycline.
- For *S. aureus* the performance was very good for both the antimicrobial susceptibility testing and the identification of the *mecA* gene. A discussion took place regarding the panel of antimicrobials to be used as some participants wanted to see more antibiotics relevant to human treatment used.
- As only dilution methods are accepted for *Campylobacter* testing the level of performance was very good with the exception of one laboratory which had recently switched to this methodology.

The proficiency test for the isolation of MRSA consisted of 8 dust specimens from a known negative pig farm, with some samples artificially contaminated with MRSA or other *staphylococci*. The participating laboratories were requested to use a defined isolation protocol and to apply PCR for the identification of the *mecA* gene. The overall results were good, with only three laboratories having more than 5% deviations.

EURL-AMR coordinated projects involving NRL's:

The EURL-AMR coordinated 3 projects in which the network of NRL's was invited to participate and a summary on the results obtained was presented at the workshop:

1. Qnr genes in *Salmonella* and *E. coli* from humans and veterinary isolates: This project consisted on the collection of retrospective data on the occurrence of quinolone resistant strains, the differentiation of strains depending on their MIC values for ciprofloxacin and nalidixic acid with the aim of screening for transferable quinolone resistance mechanisms in isolates with a plasmid mediated quinolone resistance (PMQR) phenotype, and the characterisation of resistance genes by PCR and sequencing. There were 22 participant laboratories from 17 countries with the NRL Netherlands and the EURL-AMR coordinating the study. Protocols for identification of resistance genes and positive control reference strains were distributed to the participant laboratories upon request. After screening the databases and carrying out PCR for detection of the different genes it was found that the first positive isolates were identified in 2002. PMQRs were found in 288 *Salmonella* strains from 11

of the 12 participating countries and in 20 *E. coli* strains from 4 out of the 6 participating countries. In *Salmonella*, variants *qnrB* and *qnrS1* were predominant, and were mostly associated with turkey and human isolates respectively.

2. Cut-off point for streptomycin in *Salmonella* and *E. coli*: Streptomycin resistance is regulated by *strA*, *strB* and *aadA*. More data on cut-off values for streptomycin for *E. coli* and *Salmonella* is required as EUCAST does not recommend a cut-off point and the recommended value of >16 for *E. coli* has been questioned. 217 *Salmonella* and 208 *E. coli* strains exhibiting MIC's between 4 and 32 mg/l for streptomycin were checked for the presence of the resistance genes. Results did not indicate clearly a definitive cut-off value as there were isolates with high MIC that lacked known mechanism of resistance, and also strains with resistance genes that had low MIC's.

3. ESBL prevalence: NRL's were invited to submit data on the ESBL strains encountered in years 2006 to 2009 in order to obtain a picture of levels of prevalence and mechanisms of resistance in *E. coli* and *Salmonella* in different food and animal categories. The EURL-AMR will consider the training of laboratories in the detection of resistance genes as a forthcoming activity.

Dissemination of information:

Leena Rasanen (SANCO) gave an update on the EU activities as regards foodborne AMR. She reminded the participants that there are current monitoring and reporting requirements for *Salmonella* in poultry and pigs and *Campylobacter* in broilers. Commission activities also included the baseline study in MRSA and other EU activities included those of EMEA and EFSA, i.e. opinions on MRSA and on the use of 3rd and 4th generation cephalosporins. In preparation was an opinion on macrolides and related antimicrobials and under request was a project on monitoring the sales and use of antimicrobials in animals. International activities underway included the CODEX task force on AMR and the establishment of the Transatlantic task force on AMR (TATFAR) which aims to identify areas for further cooperation between the US and the EU.

These and other presentations can be viewed at:
<http://www.crl-ar.eu/146-resentations.htm#training0308>

Report

First Meeting of the EU Working Group in Antimicrobial Resistance (AMR), Brussels, 25th May.

NRL Representative: *Dr Montserrat Gutierrez, CVRL*

AMR is in the high priority list of the newly appointed Commissioner Dalli and as a result the Commission prepared a staff working paper that provided an overview of the activities related to AMR, with a view to initiate a holistic reflection and discussion on AMR, coordinate points of view of all stakeholders and aiming to prepare a 5-year strategy in the subject. This was the first meeting of this working group and to present the subject there were a number of presentations by representatives of EFSA, EMA and the private expert, followed by a general discussion mostly on the outcome of the public consultation of the staff working paper and the necessity to include MRSA in pigs in the list of diseases in the zoonoses directive.

Elena Mazzolini (EFSA) gave a presentation on The Community Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from animals and food in the European Union in 2004 - 2007. AMR monitoring is mandatory for *Salmonella* and *Campylobacter* and voluntary for indicator organisms e.g. *E. coli* and *Enterococci*. The report, covering several years, was published by EFSA in April (<http://www.efsa.europa.eu/en/scdocs/doc/1309.pdf>) and is separate from the main Community Zoonoses report. As the results reported are obtained through a harmonised testing system (MIC) it is possible to compare results among countries. These results show large variations in resistance between MS which, in most cases, remains stable at MS level. *S. Typhimurium* shows more resistance than the other serovars. *Salmonella* isolates from pigs and cattle are more resistant than those from fowl, *E. coli* from pigs and fowl are more resistant than those from cattle. Resistance levels to quinolones and to macrolides are high in *Campylobacter* in general and *Salmonella* resistance to quinolones is high in some MS.

Pierre-Alexandre Beloil (EFSA) gave an overview of the results of the MRSA survey conducted in the EU in 2008 in breeding pig holdings in which 24 MS and Norway and Switzerland took part. A total of 1421 breeding holdings and 3176 production holdings with breeding pigs were included in the final set of results from which 145 and 416 MRSA positive samples were respectively isolated. The average prevalence was 22.4% however there was a large disparity in prevalence levels in different countries, with 9 of them including Ireland not finding any positive herds. After the molecular typing of the MRSA strains it was observed that over 92% belonged to the pig

associated type ST398, while the non-ST398 were only found in 7 countries, mostly single cases, except for Italy which presented an unusual high prevalence of strains not belonging to the ST398 lineage and including some associated with human infections. Although the final report of the study is not yet released analysis will indicate that:

- there is a greater risk of MRSA in larger holdings,
- there is vertical transmission of MRSA from breeding to production holdings and
- there is a correlation between the prevalence of MRSA and the import volumes of breeding pigs

EFSA's main recommendations include:

- further periodic monitoring of MRSA
- investigation of reasons for differences in its prevalence among MS
- investigation of the potential impacts of bio-security, managerial practices, checking of status of replacement breeding pigs in controlling MRSA.

There was a discussion on whether MRSA in pigs should be included in the list of diseases for monitoring under the zoonoses directive. MS had varied opinions on this issue. One MS pointed out that the baseline study underestimated the prevalence of MRSA as dust samples were less sensitive than nostril swabs. However expert opinion was that typing of the isolates found was a higher priority than prevalence. Some MS highlighted the need to prioritise pathogens for monitoring as the list was already large and they reminded the Commission that the zoonoses directive states that the monitoring has to reflect the epidemiological situation of the country. MS also recommended waiting until the importance of this source of infection for humans is confirmed. ECDC expressed the opinion that as MRSA was very serious concern requiring ongoing investigation.

Kari Grave and Jordi Torren (EMA) outlined the EMA mandate on European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) and other AMR activities. The Commission had asked EMA to collect data on the use of antimicrobial agents (AM) from the veterinary sector as an aid to interpreting AMR patterns and trends, to facilitate risk profiling and risk assessment regarding AMR, to allow for evaluation of control measures, to identify emerging use of AM agents, and to compare use among countries, species and veterinary vs. human. The main problems at present are the lack of harmonisation and transparency on the surveillance process. Only 10 countries are publishing reports, of them 5 (FR, GB, NL, SE, DK) collect per species, and 5 (FI, NO, DE, CH, AT) do not. EMA has analysed data from these countries and found tenfold difference between them. To compare usage it is necessary to divide total sales by the quantity of livestock per country, but it is recognised that there are a multitude of contributing factors affecting the reliability of the data including

whether it is obtained from the manufacturers or from the points of sale. EMA has established a Technical Consultative Group with experts from the 10 countries currently collecting data, and from EFSA, WHO and the EU-RL AMR to standardise data collection.

Other EMA activities outlined included consideration of methicillin resistant *Staphylococcus pseudintermedius* in dogs, monitoring AMR in animal pathogens such as *Brachyspira hyodysenteriae*, introduction of warnings in products containing fluoroquinolones and cephalosporins.

Discussion in this session included the issue of the off-label use of medicaments, the cascade principle and its potential for abuse. Several participants highlighted the major hurdles on the collection of data on sales of AM, with one proposal to make reporting mandatory at wholesaler level, similarly to what happens with pesticides.

Dik Mevius (WUR, NL) outlined the ESBL situation in the Netherlands. ESBL are enzymes that are responsible for conferring bacterial resistance to 3rd and 4th generation cephalosporins. As the genes are in plasmids problem can arise not only from the transmission of the strains among animal and human populations but also from the transmission of these plasmids between strains, mainly in the GI tract but also in the environment and in the food chain making them potentially more difficult to control. *E. coli* and *Salmonella* are the main bacteria of interest. Dutch research shows that ESBL are very prevalent in farms in NL and an estimated 10% of the ESBLs in humans are of animal origin. Off-label use of ceftiofur in chicks and pigs as thought to be responsible for this problem and Dr Mevius advocates for a strict control of the off-label and the cascade principle and perhaps the banning of the cephalosporins in animals. The differences in interpretation of the cascade principle were discussed, and the difficulties in applying prudent use of AM.

The Commission encouraged all participants to contribute to the EU public consultation "Better regulation of veterinary pharmaceuticals: how to put in place a simpler legal framework, safeguarding public and animal health while increasing the competitiveness of companies" to record their views on these and other related issues as the veterinary pharmaceuticals legislation is going to be changed.

Report

NRL Animal Proteins

Fourth Annual Workshop of the Community Reference Laboratory for Animal Proteins (CRL-AP), Turin.

NRL Representative: Dr. James Choiseul, Plant Health Laboratory

Twenty-six NRLs were represented and the presentations at this years meeting were broadly grouped into the following categories: development in PCR-based and other diagnostic methods; CRL-activities and discussions on the revision of the Regulation 152/2009 protocol.

Development in PCR-based and other diagnostic methods

Several papers were presented detailing developments in the use of PCR in the detection and speciation of animal protein in feedingstuffs. During 2009, two studies were conducted, one organised by the CRL and one done as part of the ongoing EU-funded SAFEED PAP project. Ireland participated and demonstrated a high level of competence in both studies. Overall the studies demonstrated that the PCR-protocols now available are capable of detecting animal protein in feedingstuffs at very low levels and, in addition, can be used to distinguish different protein sources (e.g. beef chicken swine, goat sheep, turkey etc). This is a distinct advantage over the microscopy method where differentiation can only be made between AP from fish and terrestrial animals. Additionally, the PCR based method can identify the origin species of muscle fibres which is not possible using light microscopy.

One of the key objectives of the studies was to determine the cut-off (or Ct) value for the PCR protocol. The Ct value is a number used by individual laboratories to determine if the product generated during the PCR reaction is reflective of the DNA content in the sample or is in fact a by-product of the PCR reaction itself. Under normal circumstances individual laboratories will determine their own Ct number. However, such self determination is not consistent with a harmonised application of the feed ban. The positive outcome of these studies was that it was possible to determine a single Ct value which could be applied by all laboratories using the PCR-protocol.

Following the success of these studies, the CRL-AP will ask the Commission to allow PCR to be authorised as an official method for AP detection. They will recommend that the test compliment the existing microscopic method. The CRL-AP envisages that training will be offered to individual NRLs for PCR testing. The CRL-AP is also planning a proficiency test for this method, in which all NRLs will be obliged to participate.

Other detection methods discussed included Near Infrared Microscopy (NIR). This method is currently used by some laboratories as a screening tool. However, it has been shown to be less accurate than microscopy and the resource costs associated with developing an NIR facility remain high.

CRL-Activities

The CRL reported on its other activities during 2009, which included the results of the proficiency test, further work on

distinguishing between terrestrial and sea mammals (i.e. whales, seals etc) in fish meal and new guidelines on the limit of detection for AP in feed. The 2009 proficiency test had 33 participating laboratories, including all 26 NRLs and 6 laboratories from outside the EU. The overall performance of the laboratories was good, with 11 laboratories, including Ireland, identifying all eight samples correctly. However, a greater number of laboratories were deemed as under performing in this PT compared with 2008. This was partly because the contamination level in the current test was lowered to 0.0025% compared with 0.1% in preceding years.

The ability to distinguish between sea and land mammals continues to be investigated by the CRL-AP. This is to address the likely contamination of fish meal by sea mammals, which pose no risk as vectors of BSE. Although it was possible to distinguish between sea and terrestrial mammals using a selection of criteria, there remains a significant level of overlap between the two and more research is proposed. The CRL-AP re-stated its need for NRLs to submit samples of sea mammals to assist it in its work.

The CRL-AP also presented a summary of their findings concerning the true limit of detection for AP in feedingstuffs. Several studies have been undertaken with the co-operation of the NRL network. These have shown that the accuracy of the NRLs is good and that the main limiting factors are now the quality of slide preparation and interpretive variation between microscopists.

Revision of Annex VI of Regulation 152/2009

The CRL-AP presented a summary of proposed changes to the above Annex submitted by the NRL network. Ireland had contributed a significant number of changes. The main changes involved a number of additional steps to be used when preparing sediments for examination, and a lowering of the current level of detection from 0.1% to 0.0025%.

NRL-AP Participation in Other Meetings

The NRL-AP participated at the UK Government Chemist dissemination event on food analysis. This meeting, which was held in London (28-29 April 2010), brought together the main UK players in regulatory policy development and cutting edge measurement science. The conference addressed developments in food safety, authenticity and analysis and was supported by The Food Standards Agency, Leatherhead Food Research, Campden BRI, LGC Standards and the Government Chemist. The overall theme of the conference was the use of DNA methods for food authenticity, laboratory validation and importance of participation in Proficiency Schemes. Presentations were given on the use of DNA in the authentication of food ingredients, case studies on the comparison of classical microscopy with molecular methods,

and the need for laboratories to implement appropriate quality assurance measures in order to produce consistent reliable data.

Report

NRL Parasites

Fifth Annual Workshop of the Community Reference Laboratory for Parasites, ISS, Rome, 3th - 4th June.

NRL Representative: Dr. Celine Mannion, CMCL

The aims of this 2-day workshop were to give an opportunity to each NRL to give a short presentation/overview of the presence/absence of parasitic foodborne zoonoses in animals and humans of their country, to present results of CRL proficiency tests and provide updates on parasitic zoonoses outbreaks.

Results of proficiency tests organised by the CRL for *Trichinella* and *Echinococcus* were reviewed, current developments in the CRL/NRLs network and the role of the commission were discussed and a number of invited guest speakers gave presentations on epidemiology and diagnosis etc. of *Cysticercus*, *Toxoplasma*, *Toxocara*, *Cryptosporidium* etc.

Bibiana Janackova (DG SANCO) gave a general overview of the role of the CRL/NRLs network focussing on the CRLs tasks and activities e.g. provision of ring tests, guidelines on validation etc. and the designation of NRLs (Regulation EC 882/2004) and their tasks e.g. collaborate with CRL, disseminate information, organise comparative tests etc. Specific issues relating to *Trichinella* testing (derogations) and new methods, accreditation of laboratories and availability of pepsin were addressed. It is possible that derogations regarding the requirement for *Trichinella* testing may be applied to *Trichinella*-free holdings within member states and some member states are in the process of applying for this. Currently serological methods for *Trichinella* testing are not accepted by the commission and if these methods are to be used for routine testing in the future they must be validated firstly by the CRL and a guideline document was provided by the CRL for discussion. Official control laboratories must be accredited, however there are derogations present in the legislation for laboratories attached to abattoirs i.e. they must have a Quality Assurance programme in place and must be pursuing accreditation. Finally the commission recognised that there have been some issues concerning availability of pepsin.

Almost all NRLs gave a short presentation / overview of the presence/absence of parasitic foodborne zoonoses in animals and humans of their country in 2009 (e.g. *Giardiasis*, *Cryptosporidiosis*, *Toxoplasmosis*, *Sarcocystosis*, *Opisthorchiasis*,

Anisakiasis, Diphyllbothriasis, Fascioliasis, Echinococcosis, Trichinosis, etc.). Many NRLs reported *Trichinella* in wild animals which appears to be endemic in these populations e.g. wild boar, fox, wolves, bear, lynx, raccoon etc. Interestingly a number of NRLs reported human cases of Trichinellosis in 2009 e.g. 433 in Bulgaria, 180 in Romania etc. Across all member states it appears that many laboratories are testing for *Trichinella* e.g. Germany has approx. 1,000 field laboratories - the NRL provides ring trials for the central laboratories which in turn oversee the regional laboratories. The U.K reported one *Trichinella*-positive fox in 2009 from Northern Ireland and highlighted the importance of auditing private testing laboratories and providing ring trials with an aim toward improving efficiency. Common areas identified for improvement were pepsin strength incorrect, microscope of poor quality, no internal QA samples etc. The majority of NRLs are proactive in providing comparative tests for *Trichinella*-testing laboratories.

A number of invited guest speakers gave presentations on various zoonotic parasites. Ronald Fayer (USDA) gave an overview of the epidemiology, diagnosis and control of *Sarcocystis* of which there are approx. 130 species. The calculation of disability-adjusted life years (DALYS) for parasitic zoonoses infections was presented by Titia Kortbeek (RIVM) using Toxoplasmosis as an example. She concluded that Toxoplasmosis is an important disease and that the disease burden is high compared to most enteric pathogens. Ronald Fayer gave an overview of the epidemiology, diagnosis and control of *Cryptosporidium* and concluded that farms are an important source of oocysts.

A presentation by Peter Deplazes (University of Zurich) on the epidemiology, diagnosis, prevention and control of *Echinococcus granulosus* highlighted the zoonotically important strains across Europe (not *E. Equinus* which is present in Ireland), some of which have seen a large increase in humans especially in Eastern Europe and the former USSR e.g. Pig strain. Various methods for diagnosis were discussed, such as Intestinal Scraping Technique (IST), ELISA, PCR, however, the sedimentation and counting technique (SCT) is still considered as the gold standard. ELISA is useful in the prepatent stages and PCR in low/high patency, however serology is often not popular due to the problems of cross-reactivity with *Taenia hydatigena* and PCR is useless unless eggs are present. He concluded by suggested that successful control programmes in sheep require vaccination, anthelmintic treatment and the culling of old sheep.

Laura Rinaldi (University of Napoli) gave an overview of the usefulness of using geospatial tools or GIS (Geographic Information System) in understanding the epidemiology and control of parasitic diseases e.g. use of geospatial tools has shown no difference in the distribution of *Trichinella spiralis* and *Trichinella britovi* across Europe except that *T. britovi* has a higher predilection for places of high altitude. Its usefulness in parasitology is in the provision of an early warning system, timing of treatments and ultimately control. A presentation by

Joke van der Giessen (RIVM) focussed on the epidemiology, diagnosis and control of *Toxocara* sp., their importance as zoonoses of pets, public awareness and concluded that responsible pet ownership is very important, however better methods are required to quantify the environmental pollution. Pierre-Alexandre Beloeil (EFSA) gave an overview of the role of EFSA in reporting parasitic zoonoses in the EU and results from 2008. There were 70 foodborne parasitic outbreaks in the EU in 2008 of which 38 were verified (37 due to *Trichinella* and 1 due to *Cryptosporidium*) - increase of 20.7% on 2007. With regard to *Trichinella* 670 cases were confirmed in humans, mainly Romania, Bulgaria and Lithuania, and *Trichinella* is most commonly detected in non-farmed wild boar > slaughter pigs > farmed wild boar. There were 891 confirmed cases of *Echinococcosis* in humans in 2008, mainly Bulgaria, Lithuania and Latvia and data was shown for approx. 17 MS that had submitted data for *Echinococcus* monitoring in farm animals. Finally further steps proposed were to harmonise schemes for monitoring and reporting of *Echinococcus*, *Trichinella*, *Cysticercus* and *Sarcocystis* in animals and foodstuffs in the EU. An invited speaker, Peter Deplazes discussed the epidemiology and diagnosis of bovine cysticercosis (*Taenia saginata*) and noted that although it results in little or no pathogenicity in humans, it can result in huge losses in cattle. In Switzerland the *T. saginata* prevalence in humans is unknown and studies have failed to shown direct transmission on farms between humans and animals. Financial losses for farmers can be as much as 50%. Other factors influencing its transmission and control are its ability to survive in the environment and the low sensitivity associated with routine meat inspection.

Gianluca Marucci (CRL) presented a report on the 2010 ringtrial on the digestion method to detect *Trichinella* larvae in pork. A brief overview of the sample preparation was presented followed by a statistical analysis of the results. For the first time 2 laboratories reported false positives, which was suggested as either due to contamination or incorrect identification of larvae. Overall no difference was observed in results whether obtained using horse or pork meat and the 2010 results confirmed the previously observed trend towards a general improvement of laboratories performance. Adriano Casulli (CRL) presented the report on the detection of *Echinococcus* sp. worms in the intestinal content of the definitive host, the aim of which was to evaluate the diagnostic skill of the NRLs. Three fox mucosa samples were prepared, one highly positive, one weakly positive and one negative. 23 NRLs participated and 22 obtained completely correct results. In future ring trials the SCT (Sedimentation and Counting Technique) technique will be used with a known worm burden.

Poland invited all NRLs present at the workshop to an international conference on *Trichinella* which they will be holding in Pulawy, Poland, 23rd - 24th September 2010.

Report**Veterinary Laboratory Service*****Trichinella* Survey**

Trichinella species (almost exclusively *Trichinella spiralis*) are nematode parasites that are significant internationally as zoonotic agents, with the pig and man being the most important hosts. Most infections can be traced back to wild animals. Commission Regulation (EC) No 2075 / 2005 lays down specific rules on official controls for *Trichinella* in meat intended for human consumption. It states that a risk-based wildlife monitoring programme should be put in place in regions where the risk of *Trichinella* in domestic pigs is officially recognised as negligible. As part of this programme, in 2008, 510 foxes were collected from locations spread across the country and delivered to the nearest Regional Veterinary Laboratory (RVL). Muscle from the forelimb, tongue, cheek and diaphragm was then harvested from each animal and digested using a pepsin/HCl solution. Following sedimentation, the digestion fluid was examined microscopically for the presence of *Trichinella* sp. larvae.

Of the 510 foxes examined in 2008 *Trichinella* larvae were found in two, one from county Cork and one from county Limerick. On a further survey in 2009, 442 foxes were examined and three (one each in counties Monaghan, Cork and Limerick) were found to be positive for *Trichinella* larvae.

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