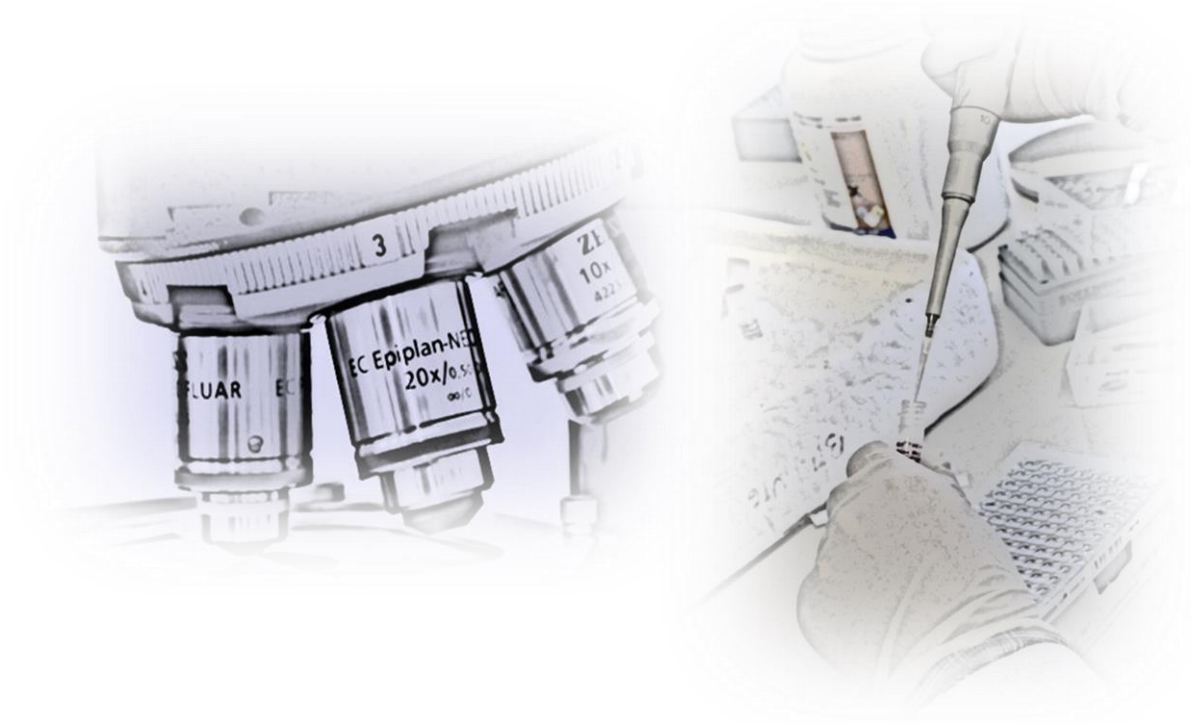
The European Union Reference Laboratory for the detection of animal proteins in feedingstuffs

Walloon Agricultural Research Centre – CRA-W (Belgium)

Annual report on the 2013 activities



Activity 1 Scientific advice & support to the European Commission (150 m/d)

1.1 Scientific advice and support to the European Commission (60 m/d):

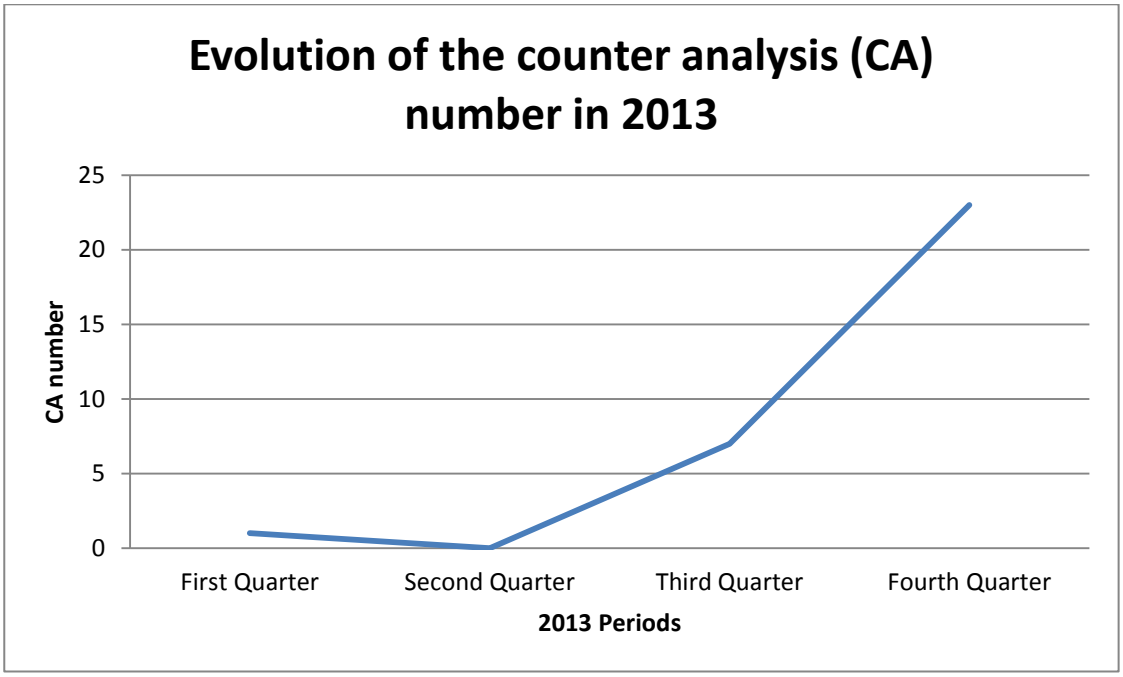
- *In relation to the development of EC feed legislation (in 2013 only tuning of the revised Annex VI of EC/152/2009 is foreseen, reflection on future extension of feed ban relaxation to pigs and poultry will be initiated [SOP preparation mainly]),*
- *Participation and scientific assistance to DG Sanco meetings (e.g SCoFCAH)*
- *Technical assistance on request of DG Sanco (e.g. scientific questions, comparison of methods, requested priority experiments). This assistance and related experiments concern issues which are not covered by other activities (Activity 4).*

- ***Meetings at the DG SANCO (7th of February, 24th of May, 12th of September)***
- ***Presence at the SCoFCAH meeting of 17th December 2013 for answering to some questions of Member States in relation to implementation of regulation 56/2013***
- ***Launching of an enquiry among the NRL network to understand the origin of the higher positive result rate with the PCR method (with notification in the rapid alert system).***
- ***Most of the samples that gave a positive result and reported in the RASFF could be reanalysed by the EURL-AP. Some additional analysis to be carried out by third parties that had been asked to help in developing new methods (e.g; looking for presence of milk proteins) were also focussed on these samples to understand the origin of the problem and also because they might be interesting samples for the development of the new methods.***
- ***On the request of the DG SANCO, EURL-AP started to record data on morphological parameters which could be used for microscopy identification of insect meals. Several insects' species were collected and processed into***

meals and micrographs were taken. A micrograph collection is under preparation and would be available on the EURL-AP intranet in early 2014.

1.2 On the request of DG SANCO or the NRLs, performance of counter-analyses on samples with disputed results (90 m/d).

- ***During 2013, several requests of official counter analysis were submitted by NRLs:***
  - ***1 sample from Member state #1, blood meal, February 2013***
  - ***3 samples from Member state #2, fish meals, July 2013***
  - ***1 sample from Member state #3, blood meal, August 2013***
  - ***1 sample from Member state #4, porcine meal, August 2013***
  - ***1 sample from Member state #4, poultry meal, August 2013***
  - ***1 sample from Member state #4, hydrolysed feather meal, August 2013***
  - ***8 samples from Member state #5, fish feeds, October 2013***
  - ***1 sample from Member state #2, complementary feed for cows, October 2013***
  - ***1 sample from Member state #2, mixture of complementary feed for cows, October 2013***
  - ***1 sample from Member state #2, complementary feed for dairy cows, October 2013***
  - ***6 samples from Member state #6, fish feeds, November 2013***
  - ***1 sample from Member state #7, fish feed, December 2013***
  - ***2 samples from Member state #7, blood meals, December 2013***
  - ***3 samples from Member state #6, fish feeds, December 2013***



- ***As expected, the number of counter analysis increased after the reintroduction of non-ruminant PAPs in aquafeed (1st of June 2013). However, the exponential increase has only begun during the last 2013 quarter and continues in the first 2014 months (cfr. the above figure)***

Activity 2 Scientific knowledge vigilance and dissemination (85 m/d)

2.1 Upon the request of the European Commission or in order to fulfil his role as EURL, participation in international scientific fora/meetings relating to the detection of animal proteins in feedstuffs (2 IAG meetings, 1 AOCS annual conference, 1 other [EFSA, EFPRA, FEFAC…]) with eventual presentations and posters to prepare for it. (missions outside the EU will get the agreement of DG Sanco) (30 m/d)

- ***The EURL-AP was present at the IAG annual meeting held in Vienna, Austria, from the 11th till the 13rd of June 2013. Four lectures were presented. The topics of the presentations were the revision of the Annex I & II of the Commission Regulation (EC) n°152/2009 concerning the sampling, the 2012 EURL-AP Microscopy Proficiency test results, the comparison study on***

different sedimentation vials and the novel method for a specific identification of ruminant processed bones.

- ***The activities of the EURL-AP were explained at the 8th meeting of the EFSA Scientific Network on BSE-TSE (EFSA, Parma, 9-10 October 2013). The members were interested to know that the EURL-AP was trying to understand what was exactly the problem with the reported positive samples found in the Member States because industry complained that too much positives were found. At that time it could not be excluded that the method was too sensitive when applied in practice even though all care was taken during the development to try to avoid this.***

2.2 Participation in the annual EURL Directors co-ordination meeting (2 m/d)

- ***This meeting was first announced for December 2013 and was finally postponed to the 17th of January 2014.***

2.3 Valorisation of EURL-AP activities (production in journals, books, help to other EURL's…) (45 m/d)

- ***The EURL-AP was solicited by the Belgian RTBF radio and television channel. A radio report about the reintroduction of the non-ruminant PAPs in aquafeed was diffused the Sunday 26th of April in "La Semaine de L'Europe" as well as a television report about the same topic in "On n'est pas des pigeons" the Friday 10th of May.***
- ***A short text about the new European animal feed regulation was also published into the 39th quarterly newsletter of the CRA-W (Summer 2013).***
- ***A paper entitled "Determination of the taxonomic origin of bone particles using fluorescence in situ hybridization (FISH)" was written. This manuscript described the protocol development of a FISH method on home-made meals and demonstrated the fitness for purpose of this technique for identifying species of bone particles regardless of an exogenous DNA contamination. This paper was finalised and submitted in December 2013.***

- ***A special edition of 'Management and Techniek', the journal of the Belgian Farming Association including a full dossier on the animal proteins issue in feed was published in 2013 with an interview of the EURL-AP related to the reintroduction of PAPs in feed for the aquaculture sector.***

2.4 Experts meeting at EURL-AP (EU experts invited for scientific discussions on current issues on PAP detection. Expert meetings are also needed for SOP management [cf 5.4]) (8 m/d)

- ***No experts meeting were organized in 2013 as it was not required.***

Activity 3 Maintenance of EURL-AP technical skills (261 m/d)

3.1 Daily and long-term management of the EURL-AP (60 m/d):

- *Team organisation and administration tasks,*
- *Maintenance of the competences of the EURL-AP team and formation of the new recruits,*
- *Preparation of the reports of activities according to the report guidelines transmitted by DG SANCO.*
- *Preparation of the new workprogram with its budget*

- ***In accordance with the comments of the revision of budget 2013 sent to the DG Sanco on 5th November 2012, the underestimated man/day (60 m/d) allocated to the sub-activity 3.1 were not sufficient for the management of the EURL-AP.***
- ***The EURL-AP participated to a FISH (Fluorescence in situ Hybridization) workshop in Porto, Portugal, in July 2013. This workshop was spread over 3 days and consisted of 2 conference days with several lectures on FISH principles and protocols, new development and new applications and one day of practical course. This training was instructive especially because it allowed interaction with specialists on practical difficulties encountered during the development of a novel FISH protocol.***

- ***Finalizing the annual report of 2012 in the beginning of 2013.***
- ***Finalizing the workprogramme of 2013 and preparation of the workprogramme 2014 with its budget.***

3.2 Maintenance and development of the sample bank (201 m/d):

- *Establishment of the specification for the EURL-AP sample bank,*
- *List of the priority needs regarding the materials to include in the sample bank,*
- *Composition analyses (PCR ,microscopy, NIR,...) of the collected samples,*
- *Maintenance of informatics tools for the appropriate management of the samples (SMS system),*
- *Collection/production of samples of animal origin in support to EURL-AP method development experiments,*
- *Storing of the samples and production of microscopic image representative of the particles,*
- *Maintenance of the sample bank.*

- ***Five home-made autoclaved bone meals from different species origin, bovine (BBM), porcine (PBM), ovine (OBM), chicken (CBM) and salmon (SBM) were prepared.***
- ***Three samples of animal meals from the Irish NRL were analysed by the EURL-AP. Due to their pure species composition, we asked to the Irish NRL to have aliquots to include in the sample bank.***
- ***Nineteen PAPs (4 poultry blood meals, 4 poultry meals, 3 feather meals, 3 pork blood meals, 3 pork meals, 2 pork graves) collected by EFPRA members and obtained in the framework of the Norwegian Research Council project SAFE-PAP (227387/E40) were also characterised by PCR.***
- ***During the second half year, efforts were centred on getting new reference samples. The samples are indeed necessary to allow the development and the validation of new analytical methods. According to the requirements, contacts were taken with industries to obtain industrial PAPs and blood***

products of pure species origins. Blood product samples, mostly of porcine origin but also some of bovine origin, were delivered. Analyses of the samples by light microscopy and PCR as well as by other methods in development were started. This will continue in the first half year of 2014. Concerning the PAPs, it was impossible to obtain pure species PAPs of ruminant origin. The other option was to ask to the industries to produce such meal in a pilot plant. Such a plant could mimic the industrial conditions as much as possible. The preparation of 5 meals of bovine, ovine, porcine, chicken and turkey origins are planned and will start in the beginning of 2014. To avoid cross-contamination, the different species were treated one by one and the suitability of the materials will be checked during the processing.

- ***To merge the results from PCR together with the light microscopy results and to perform relevant search queries on these results, the application for the samples management system (SMS) has been updated.***
- ***Part of the new samples has been analysed by NIRM and NIR imaging techniques in order to increase and update the existing databases.***

Activity 4 Development and evaluation of analytical methods (351 m/d)

4.1 Development and research on PAPs detection and characterization by light microscopy or PCR (140 m/d):

- *Contribute to the development on PAPs detection and characterization and improvement of existing methods of analysis (combination of light microscopy and PCR, complementary species specific PCR targets)*
- *Calibrants for targets in development.*

- ***The production of the calibrants for the detection of pig DNA initiated in 2012 was delayed by unexpected problems occurring during the quantification of the calibrants. These problems are most probably due to a bacterial contamination with a nucleic acid degradation as consequence. A new batch***

of calibrants was produced. In November, the number of copies in the calibrants was determined by digital PCR on the platform of the JRC-IRMM. This step was a prerequisite to finalize the in-house validation of the method.

- ***In view of the regulation revision aiming at authorization of use of non-ruminant PAP the aquafeed, several samples (91) of compound feed received from several producers origins were analysed in order to take a picture of the situation before the reintroduction of PAPs in aquafeed. These compound feeds were analysed by light microscopy and PCR (for the ruminant, bovine, porcine, poultry, sheep and fish targets). No feed was positive in light microscopy for terrestrial PAPs and 33 were positive in ruminant by PCR analysis. 32 of these positive samples were sent to the CER (Centre d'Economie Rurale) group in Marloie, Belgium for the detection of milk powder by immuno-assay. The CER group has developed Casein and BLG (beta-Lactoglobulin) ELISAs for the detection of milk and whey proteins, respectively, in foodstuffs. A single positive sample was not sent as its labelling specified that it was a "feed containing milk product". 21 of the samples were positive for at least one target. An investigations of these 21 samples revealed that 18 of them contained milk products as ingredient. The composition of 2 samples was not known and the last positive was positive for only one target (casein) and the estimated concentration was of 0.6 ppm. 35 of these 91 compound feeds were aquafeeds. As regards the PCR results of the aquafeeds only, 9 of them were positive in ruminant. One of these 9 aquafeed was positive for both ELISA targets and the others were negative for both targets. These 8 aquafeeds are still under investigation even for the presence of blood meal or blood products of ruminant origin as 2 of the samples contained blood meal or blood products.***

4.2 Development and evaluation of complementary methods (105 m/d):

- *Contribute to the development of complementary analytical methods necessary to assure the correct implementation of official methods and explorative or alternative methods (e.g. authorised ingredients vs*

prohibited PAP, influence of feed matrices, identification of sediment origin).

- *Evaluation and testing of the sequential combination of the selected successful complementary methods with a view to the SOP operational diagrams completion.*
- *Performing EURL-AP available methods or adapting them on outbreak material to make them available for the NRLs network.*

- ***The Dutch distributor of Melisa-tek kits for Benelux, Europroxima, met the EURL-AP to discuss about the follow-up of the interlaboratory study conducted by RIKILT and Nutricontrol in 2012 with two immunoassays (Melisa-tek from ELISA technologies and ReVeal from Neogen). It was repeated that the position of the EURL-AP is that the immunoassays cannot prove to have a sufficient sensitivity to be used in official controls. There is no restriction that the industry uses the Melisa-tek kit for auto-control but there is no intention of the EU Commission to add a new official method besides microscopy and PCR.***
- ***The development of the Fluorescence in situ hybridization initiated in 2012 continued in 2013. During the first half of the 2013, the protocol calibration on home-made meals was finalised and a statistical study of the method for the detection of ruminant bones was realised. As the results obtained on this type of meal were promising, efforts have then focused to adapt it on commercial meals. The first tests were performed on bovine and porcine PAPs meal available in the EURL-AP sample bank. These bone particles were not exploitable as the bone surface was covered by an opaque substance which did not allow a correct visualization of the bone lacunae and consecutively also the co-localisation. Various cleaning protocols were tested in order to remove this opaque substance but without success. As this problem seemed to be specific of these meals (industrial meals were not covered of this type of substance), contacts were taken with the industries in order to refurbish the sample bank with new batches of industrial reference***

bone meals which better reflect the classical industrial production and which will allow us to resume the method development.

- ***Concerning the complementary methods for differentiating blood products vs milk products, some contacts were established with the laboratory of cellular biochemistry and biology (URBC) of the University of Namur (UNamur). Samples (Porcine and bovine plasma powder, Whey powder, Milk powder,…) were sent in the second half of 2013 to be analysed in mass spectrometry. The aim was to identify a peptide specific for the bovine plasma powder and for the porcine plasma powder. The first analysis of the results already showed the different peptides which seem to be specific. New plasma powder batches received in the end of December will be sent to the URBC in the beginning of 2014 to be analysed and to confirm the specificity of these amino-acids sequences.***
- ***The transfer of the NIRM method to the new NIRM platform (Bruker) has been performed.***

4.3 Organization and preparation of collaborative studies for validation of methods from 4.1 (PCR pig/poultry targets) and 4.2 (when those methods are validated internally) (106 m/d)

- ***No collaborative studies have been organized on these methods since they are still under development. Man/days were absorbed by the development of methods, mainly by complementary methods.***

Activity 5 NRL network management and support (538 m/d)

5.1 Management of the Network and scientific advice (152 m/d):

- *Supply information and scientific advices (including non-official experiments or analyses of results upon NRL submission [excluding counter analyses which are included in 1.2]),*
- *Audit NRLs, coordinate on-site training on methods of analysis and assist staff from NRLs if proficiency testing reveals limited experience (on*

agreement of DG Sanco),

- *To help to develop, extend and keep in the NRLs the highest standard of technical skill and quality management under accreditation on analytical methods for detection and identification of animal proteins in feed ingredients and in feedingstuffs.*

- ***As a follow-up of the PCR implementation test 2012, two NRLs were invited at the EURL-AP facilities from the 29th till the 31st of January. During this stay, the participants performed the whole analysis from the grinding of the samples to the PCR reaction. The calibration of a PCR platform was also explained. Each participant worked totally independently on two thermocyclers and obtained good results.***
- ***The EURL-AP answered to 84 questions from the NRLs and 39 questions from other labs concerning the PCR method for the detection of ruminant PAPs. In addition, the EURL-AP answered to 9 questions from the NRLs and 20 questions from other labs concerning the horse meat detection methods.***
- ***In 2013, as a direct consequence of the results obtained from the proficiency test microscopy 2012 which showed that 5 NRLs were underperforming, two visits of NRLs were organised by the EURL-AP. Both visits happened in a very constructive and efficient way and allowed to close the cases of underperformance. Each visit included the redaction of a report that was communicated to the involved NRLs and to DG Sanco. Those reports included also recommendations and requests for the future either in terms of equipment or in terms of implementation of the methods.***
- ***On the request of the Czech Republic NRL an on-site training involving as well microscopy as PCR was organized at the facilities in Prague (see also point 5.6).***
- ***A bimonthly monitoring of the EURL-AP activities was completed and sent to the DG-SANCO.***

5.2 Communication and maintenance of information tools (36 m/d):

- *Maintenance and update of EURL website (internet/intranet) to*

disseminate and share information with NRLs and others stake holders,

- *Preparation and sending of newsletters for NRLs.*

- ***During the first quarter of 2013 the website was updated with dedicated pages related to the SOPs, the availability of the calibration kits for ruminant detection and the changes of legislation.***
- ***Furthermore, the old version of the intranet has been abandoned for a new interface integrated in the public website but with protected access for what was formerly on the intranet. It was launched since 1st February 2013. More user-friendly navigation, optimized content and to-the-point information allow the users to save time on this website. The intranet pages are only accessible, according to the user rights and password, to the members of the NRL and IAG. The micrograph collection has been revised to offer better visualisation and sorting of the pictures. A search engine was also developed allowing users to make requests on the micrograph database.***
- ***During the 12 months period, 38083 web pages from the public site were visited through 7934 visits by 3698 unique visitors coming from 93 countries and 1199 network locations throughout the world. The large increase of access to the website can be explained by the revamped website but also by the horsemeat fraud and the upload on the website (15/02/2013) of the EURL-AP recommended protocol to detect horse DNA using real-time PCR. Between 15/2/2013 and 15/3/2013, as much visited webpages and visits have been registered during 1 month as we had during 1 year in 2012. The most visited webpage after the homepage is the webpage about the method of reference and SOPs. Regarding the intranet, 1345 connexions were recorded during this period. 8 new NRL members and 3 IAG members joined the network. 6 NRL members left the network.***
- ***Monthly updates of the EURL-AP website were carried out. Maintenance tasks were provided to maintain security and confidentiality systems and to operate backup.***
- ***First issued newsletter of 2013 was prepared and posted on the intranet on***

June 2013. The 15th newsletter's content consists in the minutes of the 7th EURL-AP Annual Workshop held in Ljubljana from the 17th till the 18th of April 2013. This newsletter represents a workshop summary, including comments, decisions and brief abstract of the lectures.

- ***A second newsletter was published in December 2013. This 16th newsletter announced the next edition of the annual EURL-AP workshop that will take place in Riga in May 2014. It exposed the last development realised by the EURL-AP since the 2013 annual workshop and also explained the planned 2014 program. Several announcements for conferences and meeting were also included.***
- ***Newsletters N° 15 and N°16 are attached to this report.***

5.3 Organization of proficiency tests (1 for microscopy and 1 for PCR skills) and follow-up (179 m/d):

- *Definition (with the collaboration of the DG-Sanco) of the objectives*
- *Preparation of the proficiency tests (including homogeneity study)*
- *Invitation of the NRLs to participate*
- *Packaging and sending of the samples*
- *Collection of the results*
- *Redaction of the reports*

- ***Commission Regulations n° 51/2013 and 56/2013 give to PCR the status of official method for the detection of PAP in feed. The objective of the proficiency test was to evaluate performances of the network of 27 NRLs to detect the presence of ruminant processed animal proteins in feed using the ruminant PCR method. Due to the next coming reintroduction of the non-ruminant PAPs in aquafeed (1st of June 2013), the study focussed on the analyses of aquafeed samples.***

  Official announcement of the study was made on the 08th of February 2013 through a letter. On the 25th and 26th of February 2013, the material for the test (sets of blind samples and of calibrants) was provided to the participants

by express shipment. The Excel result report file containing the instructions, a recording sheet and a report summary as well as an updated version of the Excel file for the cut-off determination were posted on the EURL-AP intranet the 26th and the 28th of February 2013 respectively. The results had to be sent to the EURL-AP by the 5th of April 2013. The 27 participants delivered their results in due time.

Looking globally at the results sent to the EURL-AP, 92.5 % of the participating NRLs (25 labs out of 27) were able to detect correctly the presence of ruminant PAPs in aquafeed and had no false result. One NRL had one false negative result and another NRL had one false positive result. It must be stressed that the blank aquafeed used was a real-world sample with a rather high background noise for the ruminant target. It showed some risks of having false positive results during the homogeneity study. These conditions are however comparable to what a lab may be faced to in routine and the number of false positive results obtained remained nevertheless low (one lab). On the other hand, detection of a positive sample is therefore also much easier (because of the high background noise) and the lab that failed to detect it has to be considered as under-performing. The detection of ruminant PAPs in feeds in general by PCR is well implemented in the NRLs: 22 labs out of 27 NRLs (81.5 % of the NRLs) having no false result. The occurrence of false negative result is of 0.7 % (1 result out of 135 analyses) and demonstrates again the fitness of the method for the detection of ruminant PAPs. The introduction of a quality control consisting of the number of copies of the ruminant target corresponding to the Ct value of the cut-off developed to minimize the risk of false positive result improved the standardisation of the method.

As a follow-up of the proficiency test, a PCR training was organized in September.

- *The results of the EURL-AP Proficiency Test Microscopy 2012 were analysed and compiled in a report that was published on intranet in February 2013 in*

working document version. In this way, the NRL network received the report before the 2013 EURL-AP Annual Workshop. The results were presented and discussed during this Workshop in Ljubljana. After the Workshop, a final version of the report (attached to this report) was prepared and published on the EURL-AP website in July 2013. Even if the global performance was excellent for 56 % of the NRLs, if it is compared to previous studies, this percentage is low. A satisfying global performance was achieved by 26 % of the NRLs. The remaining 18 % of the NRLs were underperforming. As reported, the major problems were linked to erroneous detection of animal remains (both terrestrial and fish) in blank feed matrices – or specificity issues. False positive results are thus to be pointed out due to decisions taken on the observations of a too small number of suspicious particles or in other terms a number of particles being below the limit of detection. The changes on results interpretation in the new regulation EU/51/2013 [8] amending annex VI or regulation EC/152/2009 has been evaluated in the EURL-AP Proficiency Test Microscopy 2013. Follow up of the underperformance cases was organized.

- ***In autumn 2013, the EURL-AP designed and prepared the proficiency test for microscopy. The official announcement was made on the 6th September 2013. The preparation of the samples as well as the homogeneity study took place in September and October 2013. The homogeneity study was realised by microscopy and PCR. All the analyses were finished before sending of the sample set on the 8th November 2013. The samples were also analysed with the near-infrared microscope method (NIRM). The sample set was majorly focussed on fish feeds. The reason is the actuality related to the reintroduction of non-ruminant PAPs in feeds intended to aquaculture since the 1st of June this year. The number of samples to analyse (8) and the implementation of the revised version of Annex VI of EU regulation 152/2009 and published in EU regulation 51/2013 were the main challenges. The deadline was the 29th November 2013 and all the NRLs sent their results in***

due time. The study of the results was initiated in December 2013 and will continue in 2014 with the publication of the report. As for previous years, despite the 27 NRLs, some non-European countries were invited to participate and 5 foreign participants (Australia, China, Japan, Mexico and Norway) received the samples and returned their results.

5.4 Production of SOPs, assistance support and samples dedicated to NRL network (72 m/d):

- *Production of public SOP: definition of the objective, development, expert meeting, redaction and diffusion on internet*
- *Coordinate the preparation, reception, storage, maintenance and distribution to national reference laboratories (NRL) of samples containing animal proteins derived from different species and in particular from fish, poultry, pigs and ruminants to be used as reference materials or to carry out comparative testing. Preparation, production, calibration and distribution of plasmids as calibrants for the PCR methods (this will be taken over by the IRMM)*
- *Production of technical network SOPs (redaction and posting on intranet)*
- *Production of support (e.g. excel files for cut-off determination,...)*

- ***Five SOPs were edited by the EURL-AP on its website by end of March begin of April. Two SOPs deal with instructions related to implementation of the microscopic method of EC/51/2013. Two SOPs deal with instruction of implementation of the PCR method of EC/851/2013 for ruminant PCR. In addition a SOP strictly related to the combination of method (microscopy and PCR) was published. Another document under the form of a calculation sheet for the determination of cut-offs was made public and posted on the website. All published documents, on the exception of the SOP on operational scheme which undergone revision, are in their original V.1.0 version***
- ***At the date of the reintroduction of non-ruminant PAPs in aquafeed (1st of June 2013), the JRC-IRMM was unable to provide calibrants for the PCR detection method of ruminant DNA. The EURL-AP prepared and produced the***

plasmid solutions needed for the NRL network. Distribution was under the responsibility of the Walloon Agricultural Research Centre who coordinated this activity not only for NRLs but also for any laboratory making the request. From the 1st of July until the 4th of November, a total of 62 sets of calibrants were provided to 21 laboratories (8 NRLs – Belgium, Croatia, Czech Republik, Finland, Poland and Slovenia – and 27 public and private laboratories – 1 in France, 7 in Germany, 1 in Ireland, 10 in Italy, 7 in Spain and 1 in the Netherlands).

During the period, calibrants were also provided to Nutricontrol (Veghel, The Netherlands) and TNO Triskelion b.v. (Zeist, The Netherlands) with which EURL-AP maintains privileged relations.

- ***The EURL-AP provided to the German NRL 14 samples containing 15 g of 0.1 % w/w bovine PAP material in aquafeed. These samples helped the German NRL to implement the Ruminant PCR method in its lab network.***

5.5 Organisation of the EURL-AP workshop (36 m/d):

- *Preparation of the agenda,*
- *Invitation of the attendees,*
- *Realisation of the workshop,*
- *Minutes of the workshop.*

- ***As for the last 3 years, the annual workshop was organized outside Belgium. The 7th EURL-AP workshop was held the 17th and 18th of April 2013 in Ljubljana and its logistic organisation (meeting room renting, catering, hotel, etc), it was taken in charge by the Slovenian NRL.***
- ***The programme of the first day of meeting included the following items of presentation and discussions: The presentations of the AFSVSPP (Administration of the Republic of Slovenia for Food Safety, Veterinary Sector and Plant Protection), the National Veterinary Institute (NF-NVI) and Slovenian NRL organisation and activities, a summary of the EURL-AP 2012 activity report, the results of EURL-AP Proficiency test Microscopy 2012, the EURL-AP PCR Implementation test 2012 and the EURL-AP PCR Proficiency test***

2013. The session continued with a presentation of comparative study of sedimentation vials, summary of the next developments of PCR method for porcine and poultry targets, a presentation of the Austrian NRL about microdissection as an attractive method to distinguish between PAP and authorised ingredients like milk powder. The day concluded with the presentation of the results of PCR analysis on sediments and the presentation of the first results of a novel method for a specific identification of ruminant processed bones.

For the second day of the workshop, the presentations were firstly focused on legislative aspects with: a presentation on the future changes of legal requirements of lab samples, the legislative aspects of the review of the feed ban presented by the DG Sanco and a large discussion of the SOPs and of the requirements for new SOPs. After the break, the morning continued with the presentation of the Czech NRL and with a brief presentation of the EURL-AP involvement in the horsemeat fraud problem. The day and the workshop finished with the activity program for 2013, and the workshop conclusions. The localisation of the workshop 2014 will be in Riga (Latvia).

- ***In 2013 again, representatives of both, the microscopy method and the PCR method were invited to the workshop 2013. In most cases, the same person was the representative of the 2 methods. Minutes of the workshop were recorded in the 15th EURL-AP newsletter (attached to this report).***

5.6 Organization of specific training for microscopy and PCR (preparation, realisation at EURL-AP facilities) and other educational activities (CD, DVD, training notes update…) (63 m/d)

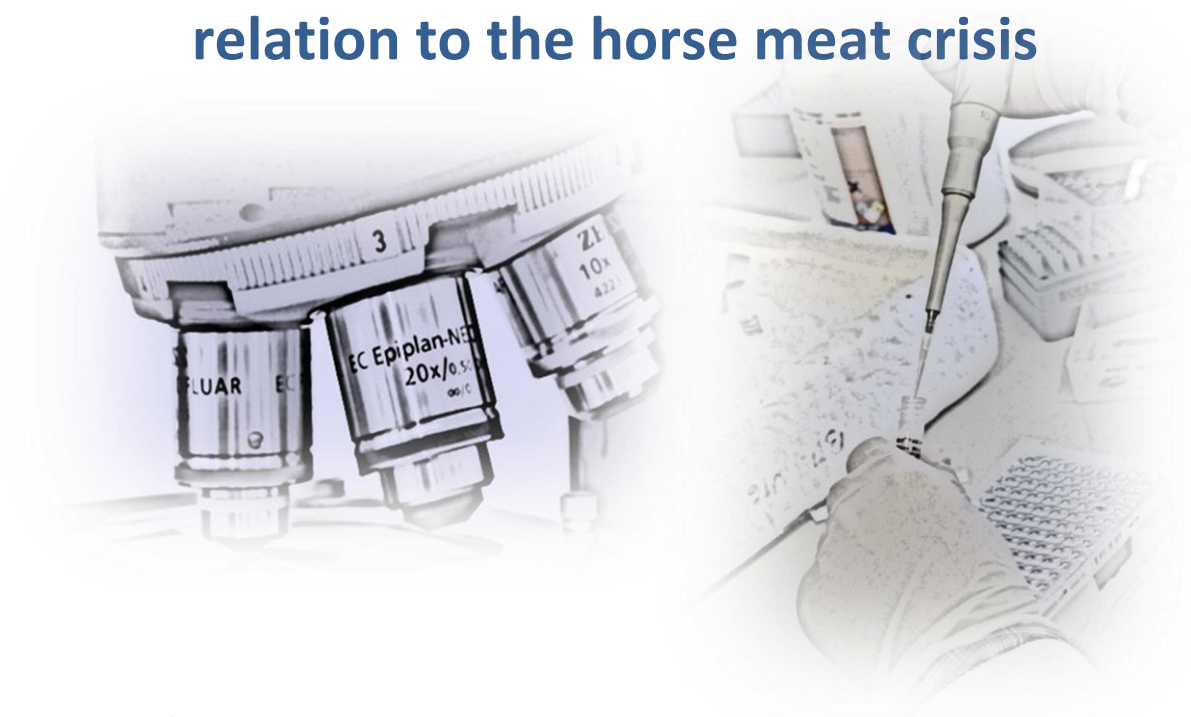
- ***As follow-up of the PCR interlaboratory studies 2012 and 2013, two trainings were specifically organised in January and September. Unlike the previous trainings, these 2 sessions were mainly dedicated to the practice according the protocols described in the official SOPs.***
- ***Training in microscopy were also organised this year. A first training with representatives from France, Latvia and Sweden took place at the EURL-AP***

facilities during February. A second training was organized in September it involved Greece, Ireland, Lithuania and Spain for microscopy and Croatia and Belgium for PCR. Finally an on-site training involving as well microscopy as PCR was organized at the facilities of the Czech Republic NRL in Prague.

The European Union Reference Laboratory for the detection of animal proteins in feedingstuffs

Walloon Agricultural Research Centre – CRA-W (Belgium)

Annex to the EURL-AP 2013 Activity report with respect to activities carried out in relation to the horse meat crisis



Inroduction

This annex to the report is based on categories of activities that are also considered for the normal workprogramme of the EURL-AP. In practice however as this additional task was a support to the Commission due to the absence of a specific EURL for the topic, all the time spent for these activities were entered in the EURL-AP timesheets under the EURL-AP activity of "scientific advice and support to the Commission". Nevertheless time spent to the horse meat crisis was flagged in a specific way and can therefore be isolated completely from the rest of the normal EURL-AP activities.

Activity 1 Scientific advice & support to the European Commission

- ***Positive response given to DG SANCO (12th of February 2013) on the fact that a PCR method is available to detect horse meat in beef***
- ***On the request of the DG SANCO (13th of February 2013) , EURL-AP agrees to make the method public***
- ***PCR method for horse detection put on the public website of EURL-AP on 15th of February 2013***
- ***Presence for scientific advice and support at the SCoFCAH meeting of 19th February 2013 in relation to recommendation 2013/99/EU.***
- ***Presence for scientific advice and support at DG-Sanco on 28th of February 2013 and 1st of March 2013 for a phone call conference with the Russian authorities in relation to a conflict between the Russian Federation and the European Union about an Austrian meat product (sausage) claimed by the Russians to contain horse meat while, if in line with the labelling, the meat content should be pure pork.***
- ***Presence for scientific advice and support at the SCoFCAH meeting of 19 April 2013 after release of results of the coordinated plan of 2013***

- ***Presence at a DG-Sanco meeting (30th of April 2013) to help to elaborate a strategy for a new monitoring plan***
- ***Preparation of the document "Stratégie pour une nouvelle campagne d'analyses en envisageant la recherche de plusieurs espèces animales dans de la viande de bœuf" as well as a questionnaire intended to the Member States (but to be distributed via DG-SANCO) to determine what are the analytical possibilities of their laboratories, to be able to work with a kind of network which is missing as there is no officially designated EURL nor designated NRLs. The document was delivered at the end of May 2013 as promised at the meeting of 30 April.***
- ***Preparation of a discussion text for the expert meeting of 26 of September 2013***
- ***Presence at the expert meeting "Miniworkshop in follow up of the horse meat crisis" of 26 of September 2013 organised by DG-SANCO in Brussels***
- ***Setting up a budget with a limited workprogramme for the second coordinated control plan (October 2013).***

Activity 2 Scientific knowledge vigilance and dissemination (85 m/d)

- ***Gathering of information on some specific techniques to be used in order to be able to give responses to technical questions of Member States (see activity 5) but also for the setting up of a new strategy as asked in activity 1 or for the discussion text linked to the expert meeting of 26 of September 2013 (activity 1).***

Activity 3 Maintenance of EURL-AP technical skills (261 m/d)

- ***This activity was not considered here.***

Activity 4 Development and evaluation of analytical methods

- ***The original PCR method made available by the EURL-AP was not quantitative but merely qualitative. As it appeared from the draft of the recommendation 2013/51/EU that a tolerance level at 1% had been considered, an addendum to the protocol had to be developed in a short time in order to make it possible to get a semi-quantitative method. This addendum was put on the EURL-AP website on 25th of February 2013***
- ***As a result of the Expert meeting of 26 of September 2013 (activity 1), it was decided that the analysis for a second coordinated control plan would be done in two steps: a first one is a screening step where laboratories check with whatever method if a threshold of 0.5 % is exceeded or not. Positive samples so identified are then checked to exceed or not the 1% tolerance level during a second step by a confirmatory laboratory that should preferably be a single laboratory per Member State. This approach should guarantee a better harmonisation of results throughout the EU than what had happened during the first coordinated plan of spring 2013 (recommendation 2013/99/EU)***

  Moreover to achieve this goal for a second coordinated control plan, the EURL-AP is asked to provide reference material to the laboratories (mainly those performing the confirmatory analysis).
- ***A first set of reference material considering three content levels of horsemeat in beef was set up : 0.5%, 1%, 2% (% in mass fraction). At that time (autumn 2013) it was expected that 40 sets gathering the several levels would be enough***

Activity 5 Network management and support

- ***As a result of the SCoFCAH meeting of 19 February 2013, the EURL-AP was asked to provide scientific advice and support to Member States in relation to technical questions with respect to methods (e.g. is the sensitivity good enough?) that would be carried out by their laboratories for the coordinated control plan linked to recommendation 2013/99/EU***
- ***Answers to a large set of technical questions have been provided to a wide variety of Member States among which mainly : Belgium, Denmark, Italy, Poland, Romania and United Kingdom. Answers have also be provided to a limited number of questions of operators in the meat sector (Belgium, France, and Portugal)***
- ***Counter-analysis on the Austrian sausage sample coming from the Russian laboratory (see also activity 1)***
  - ***Solving administrative aspects to legally import the sample with abundant mail exchanges on this issue with the Russian laboratory***
  - ***Fetching the sample at Brussels airport (16th of March 2013)***
  - ***Performing of the counter-analysis on the sample and delivery of a report to DG-SANCO***