Veterinary drugs and growth promoting agents in animal products

Annual report 2012 of the National Reference Laboratory

A.A.M. Stolker and S.S. Sterk

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Contents

Summary

This report of the National Reference Laboratory (NRL) for residues of veterinary drugs and growth promoting agents in products of animal origin according to Council Directive 96/23/EC describes the activities employed in 2012. The main tasks of the NRL are communication with Official Laboratories (OL), quality assurance of the analyses of official samples inclusive the preparation of quality control samples and the advisory function for the Competent Authority and OL. Furthermore the NRL organizes comparative tests, ensures that the OL observes the residue limits, disseminates information obtained from the European Union Reference Laboratories (EURLs) and participates in trainings organized by the Commission or by the EURLs

Communication and advices

In 2012 the NRL advised the Competent Authority on National Plan monitoring activities through the official 'Werkgroep National Plan' meetings. Furthermore, the Competent Authority was advised on the occurrence of Chloramphenicol in straw, transfer of existing MRL's from target animals to non-target animals or matrices and the development of a validation protocol for multi-residue methods. For the latter two topics the advices were done in preparation of the CCRVDF annual meeting 2012. The OL was also advised on the possible natural occurrence of Thiouracil and Prednisolone in urine of different species.

Additionally the NRL advised 3 times National Food Control Laboratories outside the Netherlands (Costa Rica, Republic of Korea and China) - on their request - about different methods of analysis for the determination of veterinary drugs.

Participation in workshops and proficiency tests

The NRL participated in workshops (3 times) and proficiency tests (5 times) organized by EURLs. The NRL participated also in 12 proficiency tests organized by other organizations like FAPAS and Progetto Trieste. In total 42 z-scores were reported of which 4 were >|2|but <4 and two results were >4 viz. 4.4 and 5.6. Each of these z-score was studied and follow up actions were formulated and performed. Most of the deviations were incidental. For the analysis of 1-testosterone in urine it was concluded that only qualitative analysis is possible. For phenylbutazone in milk: in case of positive finding accurate quantification is only possible by using the standard addition approach.

RIKILT is accredited (according to ISO 17043) for the organization of proficiency tests. In 2012 RIKILT organized for the fourth time an international proficiency test for screening and confirmation of residues of antibiotics in bovine muscle. The laboratories were asked to first carry out a screening analysis followed by a quantitative confirmatory analysis for the compounds found suspected. Thirty-eight laboratories submitted results: 33 laboratories submitted results for the screening analysis and 34 for the quantitative confirmatory part. Three laboratories characterized all three samples correctly (compliant or suspect) based on the screening analysis and indicated the correct compound groups for all samples. From the total number of 126 reported screening results (41 microbial, seven biochemical and 78 instrumental) a total number of 20 false negatives was reported. Fifteen false negatives were obtained by microbiological based screening methods and five by instrumental screening methods. A result was assigned as false negative when the analyte was not detected although it was included in the scope of the used method.

Other activities in 2012

- Special attention was paid to inform the OL regarding the analysis of beta-agonists in hair and analysis of retina.
- The NRL published -in a peer reviewed journal- a practical approach to validate a screening method. This information facilitates the OL in their laboratory validation and accreditation process.

- The NRL participates in a CEN working group on the harmonisation of microbiological based screening methods.
- Collaborations regarding research on method development/optimisation etc with OL's in Netherland and Belgium and DUCARES were continued.

1 Introduction

The European Commission is committed to protecting consumers from intolerable health hazards, which may be associated with residues of veterinary drugs or even of non-licensed or forbidden substances in animal products intended for human consumption. For this purpose legislation on veterinary drug residue control has been established as the indispensable basis of the consumer protection within the EU. The European residue legislation commits the Member States to establish an annual National Residue Control Plan (NRCP) and provides for the establishment of a hierarchically structured system of European Union Reference Laboratories (EURLs), National Reference Laboratories (NRLs) and Official Field Laboratories (OLs).

The responsibilities of the NRL are described in Council Directive 96/23/EC and include the following items:

- coordinating the work of the OLs responsible for residue analysis, in particular by coordinating the standards and methods of analysis for each residue or residue group concerned,
- assisting the competent authority in organizing the plan for monitoring residues,
- periodically organizing comparative tests for each residue or reside group assigned to them,
- ensuring that national laboratories obey the limits laid down,
- disseminating information supplied by the EURLs,
- ensuring that their staff members are able to take part in further training courses organized by the Commission or by EURLs.

This report describes the activities in 2012 of the NRL for veterinary drugs and growth promoting substances according to Council Directive 96/23/EC. It covers the groups of compounds assigned to RIKILT-NRL regarding veterinary drugs viz. nitrofurans, dapsone, nitroimidazoles, chloroform, antibiotics (including sulphonamides, quinolones, tetracyclines), anthelmintics, coccidiostats and non-steroidal anti-inflammatory drugs (NSAIDs). These groups belong to Group A6, Group B1, B2 (a, b, e) compounds as described in Council Directive 96/23/EC Annex 1. Furthermore the RIKILT-NRL includes stilbenes, stilbene derivatives, and their salts and esters, antithyroid agents, steroids, resorcyclic acid lactones (RALs, including Zeranol) and beta-agonists. These groups belong to Group A1, A2, A3, A4 and A5 compounds as described in Council Directive 96/23/EC Annex 1.

2 Methods

One of the tasks of the NRL is to communicate with the Competent Authority, OLs and other NRLs on issues regarding the control of residues of veterinary drugs. Sometimes the communication is on a regular base and sometimes on an ad hoc basis. The same is applicable to the advices given by the NRL. Sometimes advice is requested and sometimes advice is given pro-actively. The communication and advice activities employed by the NRL in 2012 are described below.

2.1 Communications

2.1.1 With the Competent Authority

On a regular basis there are meetings between the Competent Authority, The Ministry of Economic Affairs (EZ) on the content of the National Residue Monitoring Plans.

In 2012 RIKILT participated in the working group setting up the plan for monitoring residues.

During 2012 there were three meetings of this working group. Minutes are available.

2.1.2 With Official Field Laboratory

On a regular base the management of RIKILT communicates with the management of the OL. No official meetings were arranged however in 2012 more informal discussions had taken place and these were mainly focussed on the further improvement and extension of the collaboration between the two organisations.

On a regular base the analytical technicians of RIKILT communicate with the technicians of the OL. See also item 3.1.

The NRL had at least 6 technical meetings and several unofficial contacts by telephone and e-mail. Minutes are available of the official meeting.

2.1.3 With other National Laboratories

On a regular basis the management of the NRL in the field of veterinary drugs and growth promoting agents meets the management of other laboratories in this field, the so called Q3 (Quality-assurance, Quality-control, Quartet) meetings. Next to NRL and OL's, DUCARES participated in the Q3 meetings.

In 2012 the Q3 group had two official meetings at RIKILT in Wageningen, one in March and one in September. From these meetings minutes are available.

The NRL arranged a technical Q3 meeting in which in addition to the NRL and OL's, DUCARES participated. The discussion focused on the different methods available for analysis of antibiotics; microbiological methods versus instrumental methods. From the meeting minutes are available.

The NRL arranged meetings with the OL involved in milk analyses regarding the analysis of veterinary drugs in milk. The NRL prepared some quality assurance samples which were analysed by NRL and OL. Results were discussed during these meetings.

2.2 Advices

2.2.1 To the OL regarding new EU guidelines and regulations

The OL was informed about the proficiency tests and workshops organized by EURLs in Berlin, Fougères and Wageningen. One employee of the OL participated in (part of) the workshop organized by the EURL in Wageningen regarding the analyses of anabolic steroids.

2.2.2 To the Competent Authority (NVWA-Ministry of EZ)

The Competent Authority (NVWA-EZ) was advised by the NRL regarding four different items:

- Occurrence of chloramphenicol in straw. The NRL initiated in 2010 a discussion regarding the natural occurrence of Chloramphenciol. This year several samples of straw were found non-compliant for Chloramphenicol. Although straw is not really an animal feed, animals sometimes do eat from it.
- The NRL is involved in different national (collaboration with DUCARES) and international studies (FAO/IAEA programme 'Development of Analytical methods to Strengthen national Residue Control programmes for Antibiotic and Anthelmintic Veterinary Drug residues' , with the National Reference Laboratory in Uppsala, Sweden and with the European Reference laboratory for antibiotics in Fougères, France) into the natural occurrence of Chloramphenicol.
- In preparation of the annual CCRVDF (Codex working group meeting) the NLR advised the competent authority regarding the following issues: 1) transfer of MRLs from target animals to
- non-target animals, 2) MRLs for honey and 3) protocol for the validation of multi-analyte methods.
- The natural occurrence of Thiouracil, an A2 thyreostatic compound. The NRL received information on the state of the art of Thiouracil analyses and the latest articles. A proposal was made and discussed during the annual EURL workshop on how to use the latest information on non-compliant results.
- Furthermore the competent authority was informed by the NRL about the research of antibiotics in samples of surface water analysed on behalf of 'De Stichting Wakker Dier'.

3 Coördinating activities

3.1 Preparation of quality control samples

For the analytical chemical quality control in 2012, 424 quality control samples for 37 different analyses were transferred to the OL. The results of the analysis of the control samples were send to the NRL. After evaluation the results were discussed by NRL and OL and if necessary additional actions were planned/taken.

For the quality control of the microbial antibiotic screening methods, in 2012 552 quality control samples for four different tests were transferred. The results of the analysis of the control samples were send to the NRL. After evaluation the results were discussed by NRL and OL and if necessary additional actions were planned/taken.

At present screening of antibiotic residues in 'suspected' slaughter animals is carried out the OL using the NNNT (Nieuwe Nederlandse Niertest). Since a couple of years the test is supplemented with a plate test for tetracyclines to achieve adequate coverage of the latter class of compounds. In practice animals are condemned based on the outcome of these microbial screening assays. Considering the limitations of the NNNT, it is advisable to replace it by a screening approach which is better fit-for-purpose. For this reason, the NRL has recommended replacing the NNNT by the Nouws Antibiotic Test (NAT). Since the introduction of new legislation ('Wet Dieren' in force since 1-1-2013) it is 'a violation' when slaughter animals contain > MRL antibiotic residue concentrations. Considering the requirements of the 'Wet Dieren', the OL has stated that they will change the screening approach with respect to this category of slaughter animals. To maximize the detection of MRL violations, and limit the number of false positive results as much as possible, it is recommended to implement the NAT. However it is necessary (due to costs) to reduce the number of samples that have to be confirmed chemically.

The quality control samples prepared for the OL laboratory involved in milk analyses are:
- 10 samples milk containing beta-lactams
- 10 samples milk containing anthelmintics

The results of the OL were evaluated against the RIKILT reference values taking into account the method uncertainties. Next to the quality assurance samples the NRL performed a study on the stability of anthelmintics in milk samples.

The evaluation of the results obtained for the quality control samples and the results of the stability tests are communicated with the OL.

3.2 Providing analytical methods and analytical services

On request RIKILT will provide the OL's with methods of analysis and reference materials. In 2012 the following specific services were provided:
- the analytical method for benzimidazoles in liver and meat (RIKILT SOP A1089) was send to the OL.
- internal standards for the analysis of aminoglycosides were transferred to the OL.
- the OL was advised on the analysis of clencyclohexerol in hair.
- the OL was advised and trained on the analysis of retina for beta-agonists, especially on the preparation of the eye samples.
- the NRL published - in an official peer reviewed journal - the practical approach to validate a screening method. This document helps OL's with their laboratory validation and accreditation process.
- the OL was advised regarding the analysis of antibiotics in water and urine samples.
- the OL was advised regarding the analysis of clopidol in processed meat samples.

3.3 Providing information

The following documents were communicated with the OL

- Information/documentation concerning EURL workshops and proficiency tests.
- EURL information regarding the MRL for ractopamine (see attachment I).
- Q3 meeting: information regarding the possible natural occurrence of chloramphenicol and for enforcement the advice to sample the feed together with the urine.
- Q3 meeting: information regarding the possible natural occurrence of hormones like thiouracil and prednisolone and for enforcement the advice to also sample feed for Thiouracil analyses.
- All the new Maximum Residue Limits have been included in the RIKILT-NRL MRL database. The OL was informed by e-mail each time the database was edited.

Other activities regarding providing/exchanging information

- L. Stolker is a member of the Codex CCRVDF EWG (=electronic working group) cochaired by Canada and the UK (contact J. Kay). A discussion paper regarding the parameters to be set for method validation was presented during the EuroResidue VII conference (May, Egmond aan Zee, Netherlands) but additional work has to be done before there is a final version of an official paper on the 'Validation of multi-methods'.
- M. Pikkemaat participates, as the project leader, in a CEN working group on harmonisation of microbiological screening methods for detection of antibiotics. First official meeting of the working group is planned for January 2013.
- The NRL has advised the Swedish National Veterinary Institute in Uppsala (SVA) regarding the analysis of chloramphenicol in feed and straw.
- Collaboration regarding research on method development/optimisation with the FAVV laboratories in Belgium were continued.
- Collaboration with the University of Galati and the National Forensic Laboratory in Romania on the analysis of antibiotics in water were continued and L. Stolker participated as the external examiner in the PhD exam of C. Chitescu (August 15, Galati, Romania).

Activities with other laboratories

Next to the national OLs several national food laboratories from other countries asked for trainings and advice. These activities which are relevant for the NRL function are additionally financed.

- L. Stolker joined as the FAO/IAEA Agreement Holder the third annual progress meeting of projects included in the Programme CRP D5.20.36 of FAO/IAEA (Programme Development of rapid screenings method for the detection of antibiotics and anthelmintics). The project progress meeting was in Nairobi, Kenya (3-7 September). NRL chaired the meeting and gave a lecture about new developments in the analysis of antibiotics.
- S. Sterk participated in the FVO team during an audit in Bulgaria (November, 2012).
- Two scientists from Costa Rica (from the Ministry of Agriculture) were trained in the use of the LC-MS technique for veterinary drug analysis (September 3-14).
- Two scientists from South Korea (from the Animal, Plant and Fisheries Quarantine and Inspection Agency, Ministry for Food, Agriculture, Forestry and Fisheries (QIA)) were trained for 2 weeks in the use of LC-MS for veterinary drugs and and contaminants (November 12-23). A scientist from the same institute visited the NRL to discuss collaboration on the research of 'Antibiotics in the chain' (July 30 to August 3).
- Scientist from China from the National Reference Laboratory for veterinary drugs in Wuhan visited the NRL and information was exchanged about EU and Chinese residue monitoring plans (June 25-30).

4 Participating in EURL workshops

In 2012 employees of RIKILT participated to the following workshops:

- EURL-Workshop from ANSES in Fougères (June 13-15; 2012) which was dedicated to the analysis of antimicrobial residues in food and more specifically the quinoxalines. Further discussions were on the topic of antibiotics in honey, on the use of multi-analyte methods using HRMS, the need for new confirmation criteria for new analytical techniques and about proficiency testing. H. Gerritsen from the NRL participated in this workshop.
- EURL-Workshop Technical, Analytical and Statistical Issues (April 23-25;2012), BVL Berlin. The workshop focussed on the use of multi analyte methods by using LC-ToF MS. Discussions and practical session were organised. G. Bor for the NRL participated in this workshop and gave a lecture regarding 'Multi-analyte method using LC-HRMS for the analysis of veterinary drugs and pesticides in chicken meat'.
- During the workshops in Fougères and in Berlin, multi-analyte methods based on LC-HRMS were demonstrated and discussed. From the results it became clear that analyses of a large number of analytes (>50) in one single method always have suboptimal conditions for some of the individual drugs. For screening of different classes of veterinary drugs this approach is definitely of interest. However, when the interest is in targeted analysis of a specific class of compounds, more traditional LC-MS/MS is a better approach.
- Furthermore the NRL supported the need for the revision of current legislation regarding the use confirmation criteria using new advance MS techniques like HRMS.
- EURL-Workshop regarding hormones at RIKILT Wageningen from 8-10 October. The workshop focused on the theoretical and practical part of analysis of natural hormones in urine. Several NRL staff contributed to and participated in the workshop.
- Approximately, 27 experts from the NRL's - of the 27 EU member states - participated in the above mentioned workshops.

5 Participating in proficiency tests

Due to the scope of the NRL task assigned, RIKILT participated in proficiency tests organized by the EURLs, FAPAS and other international organizations. The results and (if necessary) the follow-up actions taken are described in the table below.

Table 1
PT results.

	Matrix	organized by	z-scores	Comment
antibiotics	porcine meat	ANSES		not yet reported
nitroimidazols	plasma	BVL	-0,3; -0,2; 0,09; -0,08; 0,3; -1,2; -0,5; 0,8; -0,2; 0,03	
çhloramphenicol	shrimps	FAPAS	-2,5	due to instability of enantiomer standard solution
chloramphenicol	meat	Progetto	0,6	
avermectines	meat	FAPAS	-1,5; -1,6	
steroids	urine	FAPAS	0,7; 4	alfa-nortestosterone; repeated analysis showed adequate results
quinolones	fish	ANSES	-0,65; 1,1; 0,46; -0,13	
tetracyclines	meat	FAPAS	-1,12; 0,85	
NSAIDs	milk	FAPAS	4,4; 1,6	Phenylbutazone; all participants had problems with accurate quantification of phenylbutazone
beta-agonists	hair	BVL		not yet reported
antibioitcs	meat	RIKILT	0,34; -0,2	
steroids	urine	Progetto	-1,12;-0,85; 6; -0,07; 0,95	alfa-trenbolone; re-analysis with a new standard solve the problem
A3-steroids	urine	RIKILT EURL	-1,09;-1,73; 0,22;-0,26	Including alfa-trenbolone z-scores are OK
1-testosterone	urine	RIKILT EURL	5,8; 2,6	Research PT--> conclusion 1-testosterone : no accurate quantification possible
beta-agonists	urine	Progetto	-0,56	
corticosteroids	urine	Progetto	-0,34	
coccidiostatica	ei	Progetto	-0,24	
sulphonamides	ei	Progetto	0,96; -0,09	
prednisolone	urine	FAVV		good performance no z-scores calculated

The methods involved are LC-MS/MS confirmatory methods. Most of the >|2| z-scores are incidental mistakes. For 1-testosterone it was concluded that no accurate quantification is possible. For alfa-trenbolone and chloramphenicol the standards have to be freshly prepared. Phenylbutazone in milk can only be accurate quantified by using the standard addition approach.

6 Presentations, publications and posters

6.1 NRL – Presentations

Presentation were given on the following occasions:

- Conference on 'Antibiotics in the chain' organized by the Max Rubner Institute in Karlsruhe, Germany, October 8-10. Title of the presentation: 'New approaches for detection of antibiotics in food'.

Following presentations were given at the EuroResidue VII Conference, Egmond aan Zee, The Netherlands, May 14-16.

- A. Stolker from the NRL organized a workshop for all participants to discuss the topic 'Food Safety versus Food Security'. Discussion focussed on relevance of 'zero tolerance'.
- Author W. Haasnoot; Title: Is there a future for screening technologies?

6.2 Publications, reports and posters

6.2.1 Reports, publications and posters

The following RIKILT reports have been published:

- Title: Annual report 2011 of the National Reference Laboratory; Veterinary drugs and growth promoting agents in animal products, A.A.M. Stolker and S.S. Sterk, report no. 2012.008.
- Title: Proficiency test for antibiotics and avermectines in porcine muscle; Authors: I.J.W. Elbers, B.J.A. Berendsen, M. Pikkemaat and A.A.M. Stolker, RIKILT report no. 2012.524.

Peer reviewed publications:

- A.A.M. Stolker. Application of EU guidelines for the validation of screening methods for veterinary drugs. Drug testing and analysis, 4 (2012) 28-33.
- B.J.A. Berendsen, A.A.M. Stolker, M.W.F. Nielen. Assessment of liquid chromatography-tandem mass spectrometry approaches for the analysis of ceftiofur metabolites in poultry muscle. Food Additives and Contaminants, 29 (2012) 197-207.

Posters presented at the EuroResidue VII conference:

- Author: B. Berendsen. Title: Quantitative trace analysis of a broad range of antiviral drugs in poultry muscle using column-switch Liquid Chromatography coupled to Tandem Mass Spectrometry.
- Author: I. Elbers. Title: Proficiency test for antibiotics in bovine muscle.

6.2.2 Products of interest for the NRL function (not on the expenses of the NRL project)

Publications

B.J.A. Berendsen, R. Wegh, M.L. Essers, A.A.M. Stolker, S. Weigel. Quantitative trace analysis of a broad range of antiviral drugs in poultry muscle using column-switch liquid chromatography coupled to tandem mass spectrometry. Analytical and Bioanalytical Chemistry 402 (2012) 1611-1623.

C.L.C. Chitescu, E. Oosterink, J. de Jong, A.A.M. Stolker. Utrasonic or accelerated solvent extraction followed by u-HPLC-high mass accuracy MS for screening of pharmaceuticals and fungicides in soil and plant samples. Talanta 88 (2012) 653-662.

C.L.C. Chitescu, E. Oosterink, J. de Jong, A.A.M. Stolker. Accuracte mass screening of pharmaceuticals and fungicides in water by U-HPLC-Exactive Orbitrap MS. Analytical and Bioanalytical Chemistry 403 (2012) 2997-3011.

Lectures

Lectures given at the EuroResidue VII conference:

Author B. Berendsen; Title: Column switching LC-MS/MS for the quantitative trace analysis of a broad range of antiviral drugs in poultry muscle

Author S. Ludwig. Title: Development and evaluation of a multiplex screening tool for detection of recombinant bovine somatotropin abuse.

Author M. Groot. Title: Possible contamination with clenbuterol from treated veal calves to untreated pen mates.

Author E. de Rijke. Title: Investigation of hormones in tissue using imaging DESI-MS

Author M. Blokland. Title: Endogenous steroid profiling by UPLC-MSMS and multivariate statistics for detection of natural hormone abuse in cattle

Author A. Gerssen. Title: Use of nanoUPLC and Trizaic nanoTile coupled to Time-of-Flight mass spectrometry for residues of veterinary drugs.

Posters

Posters presented at the EuroResidue VII conference:

Author I. Elbers. Title: Proficiency test for testosterone cypionate in bovine hair.

Author T. Prins. Title: Technical feasibility of forensic research to establish a relationship between syringe and injected animal.

Author B. Berendsen. Title: Quantitative trace analysis of eight chloramphenicol isomers in urine by chiral liquid chromatography coupled to tandem mass spectrometry.

Author S. Ludwig. Title: Monitoring milk for recombinant bovine somatotropin using a microsphere immunoassay-based biomarker approach.

Author S. Sterk. Title: Prednisolone A New Natural Occurring Steroid?

Author S. Ludwig. Title: Microsphere flow cytometric immunoassay for osteocalcin, a candidate biomarker for hormone abuse.

Author I. Elbers. Title: Research study for metabolite of 1-testosterone in bovine urine.

Author T. Zuidema. Title: Extent and implications of carry-over of antibiotics in porcine feed production.

Author T. Zuidema. Title: Development of a multi-class screening method for veterinary drugs and fungicides in animal feed.

Author T. Zuidema. Title: The determination of coccidiostats in egg, milk and muscle to monitor maximum levels [EC/124/2009]

Author M. Pikkemaat. Title: A simple and sensitive screening assay for antiviral neuraminidase inhibitors in poultry.

Author J. Lasaroms. Title: Is there an alternative analysis approach when you're dealing with matrix problems?

Author J. Lasaroms. Title: The determination of colistin in low level contaminated samples: Development and validation of a LC-MS/MS method.

Author A. Gerssen. Title: Web based interface for improving residue and contaminant data analysis.

Author M. Blokland. Title: Analysis for endogenous and recombinant bovine somatotropine in serum.

Author D. Doorn. Title: Presentation of a fully automated sample clean-up procedure for the isolation of growth promoters from bovine urine.

Author E. de Rijke. Title: In-vitro metabolism selective androgen receptor modulators.

Author E. de Rijke. Analysis of corticosteroids in milk.

Author M. Bienenmann-Ploum. Title: Fiveplex flow cytometric immunoassay for the simultaneous detection of six coccidiostats in feed and egg.

Author T. Bovee. Title: Screening (gluco) corticoids in cattle feed.

6.3 Organization of proficiency tests

In 2006 RIKILT has obtained accreditation for organizing proficiency tests focusing on veterinary drugs.
The following proficiency test was (in 2012) organized by RIKILT:

- Proficiency test for antibiotics and avermectines in porcine muscle - screening and confirmation. Results of this proficiency study are described in RIKILT report 2012.524 of September 2012 'Proficiency test for antibiotics in bovine meat - screening and confirmation' by I. Elbers, B.J.A. Berendsen, M. Pikkemaat and A.A.M. Stolker. The summary is presented in Annex II.

6.4 Plan for NRL activities 2013

- The quality assurance program for the OL will be continued as well as the regular meetings between the NRL, OL and the NRLs within the Netherlands (technical meetings and Q3 meeting).
- The employees of the NRL will participate in the workshops organized by the EURLs and in the organized proficiency tests (for the relevant groups of compounds).
- Collaboration and communication of the NRL with and to the OLs will be continued, including a technical meeting with participation of QLiP.

- In 2011 the NRL started with collecting/publishing the available data regarding the stability of standard reference materials. This activity will be continued.

- The outcome of the discussion on different methods of analyses for antibiotics (microbiological versus chemical), as performed within the project ‘Expertisebehoud’ will be extended to include other NL OLs and with the 3 EURL.

7 Conclusions and recommendations

From the activities described in this annual report it is concluded that the NRL for veterinary drugs and growth promoting agents in animal products has implemented all aspects as described in Council Directive 96/23/EC. The NRL communicates with, and gives advices to the (inter)national authorities and the OLs, coordinates the work of the OLs and participates in EU workshops and proficiency tests.

Furthermore the NRL organizes each year a proficiency tests (PT) which is open for (inter)national participation. However, based on the outcome of this PT, the NRL is of the opinion that further harmonization of screening for antibiotic compounds should be a priority for the EU. The NRL will participate as the project leader in a CEN working group on the harmonisation of microbiological screening methods for antibiotics.

The NRL will start a discussion regarding the natural occurrence of chloramphenicol in straw, grains and animal feed to investigate the European situation. The NRL will collaborate on this with the EURL of antibiotics in Fougères and NRL in Sweden.

References

Council Directive 96/23/EC, Official Journal L 125 , 23/05/1996 P. 0010 – 0032.

RIKILT report 2012.524, Elbers, Berendsen, Pikkemaat and Stolker, Proficiency test for antibiotics and avermectines in porcine muscle - screening and confirmation,

Annex 1 E-mail from crlvetdrug@bvl.bund.de

E-mail date: 20 September 2012 and attached was the following information:

13/09/2012

28th Session of the Coordinating Committee for Europe

Batumi, Georgia

25-28 September 2012

European Union comments on

Agenda Item 10 OTHER BUSINESS AND FUTURE WORK

Mixed Competence

Member States Vote

Under 'other business', the European Union and its Member States (EUMS) suggest a discussion on the decision making process that led to the adoption of maximum residue limits (MRLs) for ractopamine by the 35th session of the Codex Alimentarius Commission.

First of all, the EUMS would like to thank their European partners for the excellent cooperation during the 35th session of the CAC. Overall, the outcome of the CAC was satisfactory from the European point of view, with one important exception. Despite the opposition expressed by almost all of the European countries, numbering close to 45 countries, and some of the world's most populous nations, namely: China, India, Iran, Egypt, Kenya, Zimbabwe and others, the Codex MRLs for ractopamine were adopted by a very narrow vote.

The EUMS believe that the decision making process that led to the adoption of ractopamine MRLs was regrettable. As an international organisation seeking to harmonise standards across the globe, Codex should respect consensus-based decision making, one of the fundamental principles of the organisation. It is clear that for standards to be universally applicable, they also need to be universally accepted.

Taking decisions by a vote with such a narrow margin could undermine the pre-eminent role played by Codex as the world leader in setting international food safety standards. The EUMS will therefore consider ways to avoid such situations in the future.

On the substance, the EUMS reaffirm their position that an international standard for ractopamine is not justified as there remain outstanding safety and other concerns. The European Food Safety Authority has concluded that there is insufficient data upon which to make a proposal for MRLs for ractopamine and thereby risks to human health cannot be ruled out. Given its outstanding safety concerns, the European Union's current legislation will remain in place.

Annex 2 Summary report 2012.524: Proficiency test for antibiotics and avermectines in porcine muscle - screening and confirmation

The proficiency test for antibiotics and avermectins in porcine muscle was organized by RIKILT - Institute of Food Safety. The quantitative and confirmatory parts were carried out under accreditation (R013, Dutch Accreditation Board, ISO/IEC 17043). This test provides an evaluation of the methods applied for screening and quantitative confirmatory analysis of antibiotics and avermectins in porcine muscle.

For this proficiency test, three test materials were prepared:

- Porcine muscle containing ceftiofur (CEF) aimed at 250 µg/kg, desfuroylceftiofur (DFC) aimed at 500 µg/kg and desfuroylceftiofur cysteine disulfide (DCDD) aimed at 250 µg/kg;
- Porcine muscle containing ivermectin (IVM) aimed at 50 µg/kg (this level was chosen at a relevant level compared to ivermectin in fat tissue);
- Porcine muscle containing sulfadiazine (SDZ) aimed at 120 µg/kg and tulathromycin (TULA) aimed at 130 µg/kg (this level was chosen at a relevant level compared to tulathromycin in fat tissue).

The fortified materials were all prepared by spiking blank porcine muscle materials followed by cryogenic homogenization. During homogeneity testing, all materials proved to be sufficiently homogenous for proficiency testing. The stability test demonstrated that no statistically significant loss of ceftiofur and metabolites, ivermectin and tulathromycin occurred during the timescale of the proficiency test. For sulfadiazine a consequential loss occurred during the thaw-freeze cycle that was included in the stability test; this was accounted for in the calculations of the z-scores.

The participating laboratories were first asked to carry out a screening analysis. After reporting the screening results they were asked to carry out a quantitative confirmatory analysis for the compounds that were found suspect and at least for ß-lactams including cephalosporins, avermectins, macrolides and sulfonamides. Within the timeframe of the study 38 laboratories submitted results: 33 laboratories submitted results for the screening analysis and 34 for the quantitative confirmatory part.

The false positive and false negative rates were determined for all the individual laboratories and for all individual methods applied. In total, 41 microbial, seven biochemical and 78 instrumental results were reported (some laboratories carried out several different methods). In the screening part 18 false positive results and 20 false negative results were reported out of 126 reported results. A result was assigned as false negative if the antibiotic present was not detected although it was included in the scope of the laboratory. Table 1 summarizes the results of the screening methods.

In the screening part nine labs included all compound groups in their analysis (labs 2, 4, 15, 19, 20, 22, 23, 32 and 33) of which labs 15, 19 and 20 screened each material correctly for all compound groups and reported no false positive or false negative results. These three labs all applied an instrumental method. With respect to the participants' scope, which did not always include all compound groups/compounds included in this proficiency test, also labs 6, 11, 13, 16, 17, 18, 27, 28 and 38 reported correct screening results. All of these labs applied an instrumental method except for lab 18, which applied the microbial EXPLORER kit.

Detection of TULA and CEF by microbial methods relying on *B.subtilis* or *K.rhizophila* (part of EU 4 plate test, STAR test or NAT) accounted for 11 out of 20 (55% of the total amount) false negative results. Detection of SDZ by microbial methods relying on *B.subtilis* or *B.staerothermophilus*

(part of EU 4 plate test or STAR test) accounted for four false negative results (20%). Five false negative results (25%) for instrumental methods were caused by missing TULA, IVM and CEF even though these were included in the method.

Table 1

Overview of the false negative and false positive results for microbiological, biochemical and instrumental screening methods.

Material		A	B	C	
	Total	CEF/DFC/DCDD	IVM	TULA	SDZ
No. of labs submitted results	33	30	18	22	29
No. of results reported	126*	36	18	33	39
False positives	18				
Microbiological methods	8	5	2	1	
Biochemical methods	0	0	0	0	
Instrumental methods	10	3	2	5	
False negatives	20				
Microbiological methods	15	5	0	6	4
Biochemical methods	0	0	0	0	0
Instrumental methods	5	2	1	2	0

* Several labs applied more than one screening method and generated more than one result, 41 microbial, seven biochemical and 78 instrumental method were applied.

In the quantitative confirmatory part, seventeen laboratories reported quantitative results for CEF with or without its active metabolites in material A, 23 for IVM in material B, 13 for TULA in material C and 30 for SDZ in material C. For CEF the robust standard deviation was very high, so the calculated z-scores are presented for information only. For IVM 20 out of 23 laboratories obtained a satisfactory result (87%), for TULA 8 out of 13 (62%) and for SDZ 28 out of 30 (93%). Three false positive results and two false negatives were reported. One lab reported the presence of TULA in material C, but was unable to quantify it.

The analysis and reporting of CEF/CEF+DFC/all active metabolites of CEF needs additional attention because a variation in reporting the results was observed. Seventeen labs reported results for CEF, only two analysed and reported all active metabolites expressed as DFC.

Based on the results of this proficiency test it is concluded that:

- In the screening part, most false negative results are reported by labs applying microbiological methods: effort is needed to improve the effectiveness for the screening of veterinary drugs in muscle samples;
- Instrumental methods result in less false negative results, but effort is needed to include a wider range of compounds; biochemical methods did not result in false positive or negative results, but only seven methods were applied by three labs;
- The quantification of CEF and its active metabolites in porcine muscle needs attention; only two of 17 labs applied a method including all active metabolites and reported them expressed as DFC.

CEF and its active metabolites (sum of all residues) did not show an instability during the storage period, which is in contradiction to previous year's conclusions on the storage and/or stabilisation procedure of materials to be analysed for the presence of ß-lactams. In proficiency tests of 2006 (cloxacillin, ampicillin and penicillin G) and 2011 (nafcillin) stabilization procedures proved to be necessary.

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RIKILT Wageningen UR is part of the international knowledge organisation Wageningen University & Research centre. RIKILT conducts independent research into the safety and quality of food. The institute is specialised in detecting and identifying substances in food and animal feed and determining the functionality and effect of those substances.

The mission of Wageningen UR (University & Research centre) is 'To explore the potential of nature to improve the quality of life'. Within Wageningen UR, nine specialised research institutes of the DLO Foundation have joined forces with Wageningen University to help answer the most important questions in the domain of healthy food and living environment. With approximately 30 locations, 6,000 members of staff and 9,000 students, Wageningen UR is one of the leading organisations in its domain worldwide. The integral approach to problems and the cooperation between the various disciplines are at the heart of the unique Wageningen Approach.

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