

Community Reference Laboratory for Monitoring Bacteriological and Viral Contamination of Bivalve Molluscs, CEFAS, Weymouth

Technical Report for Calendar Year 2000

1 Introduction

2000 was the first complete year of operation for the CRL following designation by Council Decision 1993/313/EEC of 29 to April 1999.

2 Work plan

In March 2000, the work plan for the Community Reference Laboratory, for the year 2000, was written and submitted to the European Commission.

3 Administration

3.1 An e-mail network has been established for the dissemination of information to NRLs. NRLs of which the CRL is aware have been entered onto the e-mail database and will be kept informed of relevant information. This database will be updated and maintained as NRLs continue to be designated.

3.2 The European Commission logo was incorporated into the existing CEFAS letterhead and introduced into use during the year. The European Commission logo has the following wording alongside and underneath the CEFAS logo "European Community Reference Laboratory for monitoring bacteriological and viral contamination of bivalve molluscs."

3.3 A database of relevant scientific publications is being maintained for use by visiting scientists and to aid response to requests for information.

4 Briefings and written advice given to the Commission by the CRL

4.1 In December 2000 a briefing note was prepared by the CRL for the Commission titled "the status of live bivalve molluscs regarding possible inclusion in draft legislative requirements for *Listeria monocytogenes*." The briefing note accompanied the draft decision on the control of *Listeria monocytogenes* in ready to eat food which was discussed in a subsequent legislation working group.

- 4.2 Following an outbreak of hepatitis A virus in Spain, possibly associated with imported bivalve molluscs from Peru, the CRL provided a nine page brief to the European Commission on hepatitis A virus and methods for its detection in bivalve shellfish. The briefing article was used as a basis for discussion within the Standing Veterinary Committee.
- 4.3 Information was provided to the EU commission in February 2000 regarding an outbreak of gastroenteritis thought to originate in Scottish oysters. Information was obtained originally through the Eurosurveillance system. The CRL tested shellfish from one of the restaurants implicated in the outbreak and from samples taken from the producer.
- 4.4 Paulo Luciano, Food and Veterinary Office, Dublin. Information supplied regarding SVC report submitted to the US FDA, raw data and request from the FDA for the EU requirements for conducting sanitary surveys of shellfish growing areas within the European Community.

5 Meetings attended

- 5.1 Dr David Lees, of the CRL, attended the third meeting of the Biotoxin Reference Laboratory in Vigo, Spain, on 15-17th March, at the invitation of Cristina Laso-Sanz. This gave Dr Lees an insight into the functioning of an established European Reference Laboratory and was a useful introduction to the workings of the Community network of laboratories for biotoxins.
- 5.2 Dr Ron Lee, of the CRL, attended three meetings of the 'Working group on vibrios' in Brussels during 2000. A document has been produced by the working group for 'The Scientific Committee on Veterinary Measures relating to Public Health.' The document covers the assessment of risk to health from the presence of *Vibrio vulnificus* and *Vibrio parahaemolyticus* in raw and undercooked seafood and examines the appropriateness of setting standards for these pathogens.
- 5.3 At the request of the Commission, Dr David Lees, of the CRL, attended, as expert, a meeting in Brussels with a formal delegation from Peru. The topic of discussion was the importation of Peruvian shellfish to the EU with regard to the hepatitis A outbreak in Spain. Resulting from the meeting the CRL had a list of five actions to follow up. A Mission to Peru has been organised and is arranged for April 2001.
- 5.4 At the request of the Finish authorities Dr David Lees, of the CRL, visited Helsinki during 21st-24th March to give an invited lecture on virus contamination of shellfish at a workshop and for various scientific discussions.

5.5 The Third International Conference on Molluscan Shellfish Safety was held in Long Island, USA, 19-23 June.2000.
Kathleen Henshilwood of the CRL gave a presentation to the conference on "The use of molecular techniques for monitoring enteric virus contamination of shellfish harvesting areas."
William Dore of the CRL presented a poster titled "The use of male-specific RNA bacteriophage to model and predict virus removal by Pacific Oysters during Depuration."

5.6 The First World Water Congress of the I.A.W (International Water Association) was held in Paris, 3-7th July 2000 and was attended by Dr. David Lees and Dr. Ron Lee of the CRL. The following people from the CRL also attended and gave poster presentations at the conference :

Lisa Cross

"The development of quantitative molecular assays for determination of enteric viral load within environmental samples."

William Dore

"Levels of F-specific RNA bacteriophages in molluscan bivalve shellfish from commercial harvesting areas."

Roger Glover

"Factors influencing faecal contamination of shellfisheries as determined by statistical analysis of faecal indicator concentration."

Kathleen Henshilwood

"Monitoring the marine environment for small round structured viruses (SRSVs): a new approach to combating the transmission of these viruses by molluscan shellfish."

Richard Pepperell

"Progress towards a simplified method for detection of enteric viral pathogens in shellfish by RT-PCR."

5.7 Dr Ron Lee, of the CRL, attended a WEFTA meeting, (West European Fish Technologists Association), in September at Lyngby near Copenhagen. Dr Ron Lee has been attending these annual meetings for the past four years and has been the acting rapporteur for the last two years. The meetings exchange information on fish and shellfish microbiology in the areas of quality and public health. The meetings are attended by representatives from most European countries and a report of each meeting is produced for the participants. Currently Dr Ron Lee is compiling a report covering details for collaborative work on vibrios to be carried out among the participants of this working group.

6 Practical work carried out by the CRL

6.1 Outbreaks investigated by the CRL included samples from New Zealand, Denmark, UK and Ireland.

6.2 A sample of Peruvian clams was sent to the CRL from Pescados SRL', Peru, for Hepatitis A virus analysis. Results were returned to Salvador Perez (Pescados SRL), Antonio Mayorga (Sanidad Exterior, Puerto de Valencia) and Henri Belveze (European Commission). Communication was also made with Dr Guy Carvajal, Scientific Adviser, Ministry of Health, Lima, Peru.

Dr David Lees informed Christina Laso-Sanz of the situation and sent a copy of a paper by Professor Albert Bosch, Barcelona University, who had written up the preliminary details of the outbreak in Valencia. He also sent the report written by the competent organisation of the autonomous region of Valencia titled 'Cause of the contamination of coquinas from Peru.'

6.3 Dr Ron Lee, of the CRL, carried out vibrio testing for Beatriz Ferro Soto, (Department of Microbiology and Pathology, Centro de Control da Calidade do Medio Marino, Galicia, Spain), during November and December 2000.

Nineteen samples of vibrio were sent to the CRL for species identification. The results were returned to Beatriz Ferro Soto who was then able to use them for comparison with the results obtained in-house.

6.4 Andrew Younger, of the CRL, and Daniel Gadd, of CEFAS, visited IFREMER, Nantes, France, 6th November 2000, to meet with Benoit Beliaeff (statistician) and Patrick Camus (modeller). The meeting covered options for the potential use of statistical methods relating to the shellfish harvesting area classification systems and details of modelling techniques used in the prediction and assessment of contaminatory effects in shellfisheries.

7 Written advice provided to NRLs, prospective NRLs, and third parties

Written advice has been given to a number of prospective NRL's on the requirements of Council Decision 1999/313/EC and the type of laboratory expertise, laboratory facilities, and scientific staff that would be required to meet the Council Decision requirements. Written advice on other topics arising from specific queries has also been given.

Advice was provided by the CRL to the following:-

7.1 Dr Eric Dubois from Agence Francaise de Securite Sanitaire des Aliments, France.

- 7.2** Dr Neal Dunne, Biological and Environmental Services Unit (BESU), Cork, Ireland. There is currently a proposal for the CRL to collaborate with BESU on viral detection techniques in shellfish as part of a British Council/Enterprise Ireland Research Visit Scheme.
- 7.3** Professor Thirty and Professor Daube (Service de Virologie, Faculte de Medecine, Veterinaire, Belgium) regarding indicator methods for Directive 91/492/EEC and methods for the detection of viruses in shellfish.
- 7.4** Mr Alan Reilly, (Food Safety Authority of Ireland), regarding Council Decision 1999/313/EEC, viruses and the UK standard method for monitoring *E.coli* in shellfish.
- 7.5** Dr Ester Sunen, (Universidad del Pais Vasco, Spain), on the control of viruses in the clam industry and the protocol for virus extraction from the digestive gland.
- 7.6** Dr Armando Hung (University of San Marcos, Lima, Peru) on the methods used at the CRL for the detection of viruses in shellfish.
- 7.7** Ms Gertrude Lanzon, Scientific Officer, Public Health Laboratory, Valletta, Malta.
Information was sent on virological techniques for the detection and control of viral contaminants, standard methods for *E.coli* and salmonella analysis and contact details for participation in external quality assurance schemes run by the Public Health Laboratory in England.
- 7.8** Dr Weise, Director, Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV), NRL, Germany. Information was sent on virological techniques for the detection and control of viral contaminants and standard methods for *E.coli* and salmonella analysis. Advice was given regarding *Vibrio* analyses and the responsibilities for NRLs.
- 7.9** Communication with Dr William Burkhardt III, Senior Regulatory Research Officer, FDA, Alabama, USA, regarding potential collaboration on FRNA (male-specific) bacteriophage and an exchange of historical data between the European Union and the United States.
Cristina Laso-Sanz (EU Commission) was informed of the suggestion made by CRL to host an international workshop on viruses as a way of taking forward collaboration between the EU and the FDA.

- 7.10** There is a proposal for the CRL to collaborate with the University of Aberdeen (Dr Andy Porter) and the Scottish Marine Laboratory (Dr Susan Gallacher) on new molecular methods for the detection of enteric viruses.
- 7.11** Advice on methods of viral detection was given to Dr Jesus Romalde of the University of Santiago de Compostela.
- 7.12** Advice on methods of viral detection was given to Kerry Jackson of the New South Wales Shellfish Quality Assurance Program, Australia.
- 7.13** A discussion on methods of viral detection took place between the CRL and Carl-Henrik von Bonsdorff of Helsinki University.

8 Training provided to NRLs (and prospective NRLs)

During the year 2000 a number of visitors from NRLs and Member State Competent Authorities were received at the CEFAS Weymouth laboratory for discussions on various aspects of viral and bacterial contaminants of shellfish.

Visits to the CRL laboratory, Weymouth, included :-

- 8.1** Four colleagues from the Marine Institute, Dublin specifically interested in shellfish viruses. 11-12th April.
- 8.2** Dr Alexandra Scipioni from the Service de Virologie, Faculte de Medecine Veterinaire, Liege, Belgium visited 28th February - 3rd March. The laboratory was later nominated as the Belgian National Reference Laboratory for monitoring bacteriological and viral contamination of bivalve molluscs. Dr Scipioni met with the following people at the CRL : Dr David Lees to discuss the role and remit of National Reference Laboratories, Dr Ron Lee, Bill Dore and Andrew Younger to discuss the classification of harvesting sites and with Kathy Henshilwood to discuss the virological techniques used at the CRL. Dr Scipioni also spent time in the bacteriology laboratory to learn the methods of analyses for *E.coli*, salmonella and F+ bacteriophage analyses.
- 8.3** Michael O'Conneide and Joe Silke from the Marine Institute, Dublin, Ireland, visited in April. They met with Dr David Lees to discuss the role and remit of National Reference Laboratories and Joe Silke met with Julie Younger to discuss requirements for EN45000 compliance. They also met with other CRL staff members to discuss shellfish viruses and virology techniques.

- 8.4** Dr Andy Petersen (Scientific Director) and Kevin Callanan from the Biological and Environmental Services Unit (BESU), Cork, Ireland, visited 9th August. They discussed the role and remit of National Reference Laboratories under Council Decision 1999/313/EC. Some areas of common interest were also identified which could be used for collaborative purposes in the future.
- 8.5** Charlotte Schultz from the Danish Veterinary and Food Administration, NRL in Denmark visited 4-8th December. Charlotte met with the following people at the CRL: Dr David Lees to discuss the role and remit of National Reference Laboratories, Dr Ron Lee, Bill Dore and Andrew Younger to discuss the classification of harvesting sites, Kathy Henshilwood to discuss the virological techniques used at the CRL and Dr Rachel Rangdale to discuss the methods for *E.coli* and F+ bacteriophage analyses. Charlotte spent time in the bacteriology laboratory to learn the methods of analyses for *E.coli* and F+ bacteriophage and in the molecular laboratory to learn the methods for the virological techniques.

9 EN 45000 criteria

An initial pre-assessment visit from the United Kingdom Accreditation Service (UKAS) was made to the CRL, Weymouth, in July 2000. The following analytical methods were investigated:

'Enumeration of male-specific RNA bacteriophages in molluscan bivalve shellfish',

'Enumeration of *Escherichia coli* in molluscan bivalve shellfish'

'Examination of shellfish for *Salmonella* spp. other than *Salmonella typhi*'.

A follow up visit to assess the laboratory for these analyses is arranged for April 2001.

10 Comparative testing and ring trials

A meeting took place in February 2000 between CEFAS and PHLS in which the development of PHLS/CEFAS partnership for European Shellfish EQA was discussed. It was noted that a number of countries with shellfish industries did not participate in the shellfish scheme, namely Portugal, Eire, Spain, Denmark and the Netherlands. Some European countries have approached PHLS with regards to a comparative testing arrangement for shellfish testing laboratories.

The meeting also pointed out that the European scheme would need to be self-financing and thus participating laboratories would need to pay a fee as for the present shellfish scheme. It was noted that EU funding might be available for the initial development of a scheme.

11 Invitations

- 11.1** Food Safety Authority of Ireland inviting Dr David Lees to present a short review of viruses in shellfish - February 2001.
- 11.2** 4th International Conference on Molluscan Shellfish Safety inviting Dr David Lees to present in the subject area of microbiological contaminants including viruses, depuration, water quality and harvest area monitoring - June 4-8, 2002.