The 2012 Annual Meeting of National Reference Laboratories for Mollusc Diseases was held in IFREMER in Nantes on the 14th and 15th of March 2012. In total, 39 participants from 18 European countries (Bulgaria, Croatia, Denmark, France, Germany, Greece, Ireland, Italy, Lithuania, Norway, Poland, Portugal, Romania, Slovenia, Spain, Sweden, The Netherlands, United-Kingdom), and experts from Australia, Spain, United Kingdom and one representative from DG-SANCO attended the meeting.

The Annual Meeting included eight sessions: 1/ Current epidemiological situation in the Member States 2/ Implementation of the Directive 2006/088/EC 3/ *Crassostrea gigas* mortality outbreaks 4/ How should we use Real Time PCR 5/ Infections with two pathogens affecting molluscs in Australia 6/Risk based surveillance approach 7/Information on other pathogens 8/ EURL day life activities and related projects. The summary has been prepared by the EURL.

Summary

Follow highlights of the discussions, expert opinion and recommendations formulated during the 2012 Annual Meeting of National Reference Laboratories (NRLs) for Diseases of Molluscs:

In 2011, major **epidemiological changes** in EU were (1) the detection of ***Marteilla refringens*** in asymptomatic mussels *Mytilus edulis* from Cornwall, in UK (2) the confirmation of the presence of ***Bonamia exitiosa*** in flat oysters *Ostrea edulis* collected in December 2010 in Cornwall, UK; this parasite was not detected in 2011 (3) the detection and characterization of **new *Mikrocytos* species** in closed association with mortalities of *Donax trunculus* like in 2010 but also in archived material from 2008 in France (4) the detection of **OsHV-1** showing sequences different from OsHV-1 (ref) and OsHV-1 µvar in larvae of flat oysters in Sweden and oyster larvae (species not determined) in The Netherlands. Compared to last years, more Member States included mussels in their surveillance programme for marteiliosis. Ireland observed less *Crassostrea gigas* mortality outbreaks compared to the past two years.

Following the **detection of *B. exitiosa*** in flat oysters from Spain in 2007 and from Italy in 2008, a working programme was proposed at the European level to find out the actual spread of this parasite previously considered exotic to EU. Results from this working programme including its detection in the context of mortality event or suspicion case suggest that *B. exitiosa* is present in at least four Member States (Spain, Italy, France and UK). Mixed- infections (with B. *ostreae)* at the same location and even in the same oyster were observed which does not facilitate investigation of their respective impact on flat oyster populations. The analysis of sequences including ITS-1 and ITS-2 reveals low polymorphism among European B. *exitiosa.* The wide spread of this parasite previously considered exotic in EU raises some questions regarding the origin of its presence in Europe.

A new **Animal health law,** covering all vertebrate and invertebrate animals, including aquatic animals, is presently under preparation. The rules applicable to aquatic animals will be aligned with those applicable to terrestrial animals to the extent this is appropriate taking into account the specificities of aquaculture production and the principles of Directive 2006/88/EC.

Pending the adoption and implementation of the Animal Health Law, Directive 2006/88/EC remains in force and the efforts in reinforcing the national implementation of that Directive should continue. All aquaculture production businesses and authorised processing establishments are to be **authorised,** and Member States shall establish a public available register containing information on each farm, including information on the species kept and the health status of the farms as regards the diseases listed in Annex IV to Council Directive 2006/88/EC. The link to Member States and EFTA states' web pages with register of aquaculture production businesses and authorised processing establishments in their territory can be found

on the following link: http://ec.europa.eu/food/animal/liveanimals/aquaculture/register_aquaculture_establishments_en.htm .

There are presently seven Member States producing molluscs which have established such a list. Next step is to **categorise** each of these aquaculture production businesses according to their status regarding diseases (I- Disease free; II-Surveillance programme; III-Undetermined, IV-Eradication programme, V-Infected). Few Member States have started health categorisation.

Obligations under EU legislation require MS to have a surveillance system in place that is able to detect new and emerging diseases, listed diseases and the detection of abnormal mortality. The detection of a new or introduction disease in farmed populations relies on passive surveillance (i.e. reporting by the farmer). **Sensitivity of the detection of mortality** events is thus a key quality indicator which appears influenced by outbreaks occurrence and by implementation of financial incentive for mortality reporting.

Furthermore in order to maximise the efficiency of the surveillance system for a fixed level of resource, **risk based approaches** should be used and require ranking shellfish areas based on the likelihood of disease introduction and spread. The key factors that should be taken into account in risk ranking shellfish production areas include live animal movements for aquaculture or human consumption and introduction via water.

Following the abnormal *Crassostrea gigas* mortality events associated with the detection of OsHV-1 µvar in Europe since 2008, the Commission Regulation (EU) No 175/2010 was accepted by the European Commission and then replaced by the Decision 2011/187/EC. This decision concerns the approval of **national measures for preventing the introduction of OsHV-1 µvar** into certain areas of Ireland and United Kingdom. After two years of surveillance including 47 sample sites in UK, only a single site has tested positive for OsHV-1 µVar. By the end of 2011, 26 bays in Ireland had been identified as positive for the virus and 17 areas remained in the surveillance programme. Different diagnostic approach and new diagnostic tools enabling the indirect (PCR- RFLP) or direct (LNA probe based taqman assay) **detection of OsHV-1 µvar** have recently been developed and could be useful for the surveillance programme or in case of mortality outbreak.

The recent emergence of **OsHV-1 µvar in Australia** has lead Australian competent authority to implement control measures restricting the spread of the disease. Since its first detection in late 2010 no other production site has shown *Crassostrea gigas* abnormal mortality. Sequencing of the virus detected in Australia is awaited in order to compare it with OsHV-1 µvar detected in Europe. Another member of the Herpesviridae family, causing **abalone viral ganglioneuritis** (AVG) has recently (late 2005 onwards) been identified and characterized in farmed and wild abalone showing mortality in Australia. The disease is listed by the World Organisation for Animal Health (OIE) in the Aquatic Animal Health Code (2011) under the following name "Infection with abalone herpes-like virus". Some variants of this virus have also been described.

***Vibrio* species a**re frequently detected in association with mortality of *Crassostrea gigas* in addition to OsHV-1 µvar in the case of *V. splendidus* or alone in the case of *Vibrio aestuarianus.* However the surveillance of these bacteria is not harmonized at the EU level and might present some limits. Molecular tools presently available for the detection of V. *splendidus* are not specific enough and their use in the context of investigation of mortality outbreak is not relevant. Moreover, considering the difficulty to isolate V. *aestuarianus* (specific temperature and slow growth) this species is very probably underdetected.

The recent detection of *Marteilia refringens* in mussels in Sweden and UK suggests that the geographic distribution of this parasite is wider and further north than previously considered.

Moreover, recent works have shown that other congeneric species might occur in Europe. Indeed, a **new *Marteilia* sp. type C** was detected and characterized in **cockles** *Cerastoderma edule* from Catalonia in Spain.

In **Australia,** another member of the genus *Marteilia,* ***M. sydneyi*** is the subject of many studies because of its impact on Sydney rock oyster, ***Saccostrea glomerata,*** populations.

Management of the disease, named QX disease (for Queensland unknown disease prior to the discovery of *M. sydneyi)),* has notably been hampered by the complicated life cycle of *M. sydneyi.* The availability of hatchery-produced QX-resistant *S. glomerata* should ensure the sustainable production of this native oyster species in the future.

More and more laboratories use Real Time PCR for disease surveillance activities. The Dutch Reference Laboratory for mollusc diseases has recently carried out some convincing tests to evaluate the possibility to use **Real Time PCR for routine screening** of *Bonamia ostreae* and *Marteilia refringens.* However, based on results from the **interlaboratory comparison** organised by the EURL in 2011 to test the competency of NRLs to detect OsHV-1 using Real Time PCR, it seems that such assay is subjected to **variability** depending on laboratory conditions including PCR machines and reactives. This variability did not influence so much qualitative results which were good (75% of participants had 95,6% of good responses or more) but influenced quantitative results. Moreover, the choice of a **cut off value** appeared crucial in the interpretation of the results for negative or lightly infected samples. The selection of a cut off value should be done carefully and should **fit a diagnostic purpose.**

Considering the increase of the use of PCR based methods and the continuous development of molecular diagnostic tools for the detection of mollusc pathogens, it appears relevant to propose a **working programme** at the EU level in order to collate specific data on PCR assays used in the NRLs and to compare the most widely used ones among the NRLs. After organising a survey using a questionnaire sent through the NRLs network, the EURL suggests to establish collectively a **harmonized approach to compare PCR assays** and finally to organise and perform interlaboratory study for most commonly used PCR assays. In a first step, this approach could be concerned the parasites of *Bonamia* and *Marteilia* genus.

The **EURL website** http://www.eurl-mollusc.eu/ is continuously updated. Since last year, NRLs can use the software Mscope through the EURL website. **Mscope** enables access to scanned histological slides including all the previous ring test slides and a set of reference ones and allows self evaluation. The activities of the EURL in 2011 are presented through the website sections "main activities" and "scientific activities".

The EURL proposes to plan the **next annual meeting** for the beginning of 2013 (March) in association with a **Technical Workshop** at the Ifremer facilities in La Tremblade, France. NRLs are invited to propose topics on which they are working or for which they need information and/or training as soon as possible.