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PANDA

Permanent network to strengthen expertise on infectious diseases of aquaculture species and scientific advice to EU policy (PANDA)

Coordination Action

Scientific support to policies

**Deliverable 9:** Report identifying how to achieve harmonised implementation throughout Europe of the best diagnostic methods for the main disease hazards in aquaculture.

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## CONTENTS

**EXECUTIVE SUMMARY** ................................................................. 3

1. **INTRODUCTION** ................................................................................................. 4
   1.1 Aim of D9 of PANDA WP4 ................................................................. 4
   1.2 Methods used to gather information .................................................... 4
   1.3 Structure of the report and method to use it ........................................ 4
   1.4 General remarks and links with other WPs of PANDA ................. 4

2. **HOW TO ACHIEVE HARMONIZED IMPLEMENTATION IN EUROPE?** ....... 6
   2.1 Why harmonization? Background and aim ............................................ 6
   2.2 Recommendations to achieve harmonized implementation ............... 6
   2.3 Gaps in knowledge, training etc ............................................................ 10

3. **CONCLUSIONS** .................................................................................................. 14

4. **ACKNOWLEDGEMENTS** .................................................................................. 15
EXECUTIVE SUMMARY

In the D8 report of PANDA, the current best methods were investigated, for rapid and accurate detection of the main disease hazards in aquaculture, and evaluated. In this report, D9, recommendations are made, based on the evaluation of D8, to achieve harmonised implementation throughout Europe of the best diagnostic methods for the main disease hazards in aquaculture.

Related to the new EC Directive 2006/88/EC with its extended disease lists, and the WP2 list, there is an urgent need for acquiring expertise and in training on diagnosis and detection of various exotic diseases/pathogens of aquaculture species, as concluded from the WP4 report of PANDA. Especially, the diagnosis of newly EC-listed diseases Epizootic Haematopoietic Necrosis (EHN), Koi Herpes Virus Disease (KHVD), Epizootic Ulcerative Syndrome (EUS) of fish, and the viral diseases of crustaceans Yellow head, White Spot and Taura need urgent training for at laboratories within the EC. Additionally, the task force of WP4 is convinced that the diagnosis of the non-listed crustacean hazard caused by Aphanomyces astaci needs urgent attention at laboratories in the EC. The amphibian pathogens RANA virus and Batrachochytrium dendrobatidis are new to most laboratories. Appointment of a CRL by the EC is necessary, after which certain laboratories should get expertise and skills in testing via training.

With the new lists of diseases of the EC and WP2 of PANDA, the tasks to achieve harmonised implementation throughout Europe of the best methods are extended for several responsible bodies: The European Commission, Community Reference Laboratory, and the National Reference Laboratory with their government will have to put much effort and money, using the PANDA network and world wide experts, to get the expertise into Europe and to the CRL’s, NRL’s and regional labs. Priorities have to be made in the whole process, and therefore ad hoc expert groups need to be appointed first. In this way, the PANDA network can be further used.
1. INTRODUCTION

1.1 Aim of D9 of PANDA WP4
Deliverable 9 (D9) is this report, identifying how to achieve harmonised implementation throughout Europe of the best diagnostic methods for the main disease hazards. It includes the needs for strengthening knowledge and technical skills to achieve harmonised application within the EU for the current best diagnostic methods identified. Additionally, recommendations for guidelines and policy/legislation options are given, with regards to harmonised application of current best practices for rapid diagnosis.

As Directive 2006/88/EC is in place from 2008, this means various new listed diseases/pathogens for aquaculture for Europe. Apart from viruses, bacteria, parasites and fungi are added to the list compared to Directive 91/67/EC. It means more different techniques to be used to cover the diagnosis of these, partly exotic diseases. Inevitably this means an extension of the tasks of the Community Reference Laboratories and National Reference Laboratories in aquaculture diseases. In the last decade, many new member states have accessed the EC. Their tasks will also be extended. Overall, the above facts will result in many training needs as consequence.

1.2 Methods used to gather information
Deliverable 9 (D9) was discussed in March 2007 at the plenary PANDA workshop at Weymouth, after facts for D8 (the current best methods for rapid and accurate detection of the main disease hazards and requirements for improvements and their eventual standardisation and validation) had been investigated. Current methods were discussed based on the expertise of each work package member, and in the light of recommendations by the OIE, and the new EC Directive 2006/88/EC.

1.3 Structure of the report and method to use it
This report can be seen as an Annex to the report of Deliverable 8 (D8), identifying the current best methods for rapid and accurate detection of the main disease hazards and requirements for improvements and their eventual standardisation and validation. The gaps and needs identified in D8 were translated to recommendations, and those are given in this D9-report in Chapter 3.

1.4 General remarks and links with other WPs of PANDA
The WP2 list contains many diseases/pathogens which are exotic to Europe. It means, knowledge on these diseases, and their specific diagnostic techniques are so far often only present at one laboratory or even none within Europe. As a consequence, this WP2 list of hazards, the lists of the new EC Directive, and the list of the Aquatic Animal Health Code of the OIE (2007) are overlapping. Europe starts from scratch with diagnosis of some of these diseases.

The WP4 task force consists of a small group of European multidisciplinary aquatic disease experts, each with their own subjective view on the current plan to achieve harmonisation throughout Europe of the best diagnostic methods for the main disease hazards. This implicates, that views on the harmonisation are subjective and for the present situation. The views may change in time.
The training needs related to WP4 were communicated with WP6. The training needs and recommendations of WP4 can be found both in the D9 of WP4 and in D11.
2. HOW TO ACHIEVE HARMONIZED IMPLEMENTATION IN EUROPE?

2.1 Why harmonization? Background and aim
With the globalization, there is more and more international trade in live aquaculture animals. This trade implies high risks of introducing and spreading aquaculture diseases from one to another country. The EC has good legislation to be able to trade relatively safe, by the current Aquaculture Directive 91/67/EC, and by implementing the EC Directive 2006/88/EC.

Related to diagnosis of disease and detection of pathogens in aquaculture, member states should be confident about their test methods: The diagnostic test result of a disease should be the same in one or another member state, so, their tests should have the same Quality Assurance level or validation level.

Community Reference Laboratories (CRL) for Fish Diseases (DTU, Århus, Denmark) and Mollusc Diseases (IFREMER, La Tremblade, France) respectively function in educating the National Reference Laboratories (NRL) already for years on the current listed diseases: they organize Annual NRL meetings, and annual or bi-annual ring tests for NRL’s. Additionally, the OIE Reference Laboratory for Koi Herpes Virus Disease (CEFAS, Weymouth, UK) organizes ring tests for PCR testing of Koi Herpes Virus.

Related to fish diseases, the education of NRL’s by the CRL and CEFAS is specialized to viruses, present in Europe. However, in the new EC Directive 2006/88/EC, a fungus and 2 exotic viruses are added to the lists of fish diseases. Additionally, the mollusc diseases/pathogens list is changed, and various crustacean and amphibian diseases/pathogens are listed for the first time. This means an extension of tasks of all NRL’s, and the CRL’s for Fish Diseases and Mollusc Diseases, respectively. All labs need to be prepared to diagnose these diseases, or delegate diagnosis to another national laboratory or to the NRL of another member state. According to the EC Directive 2006/88/EC, also a CRL for Crustacean Diseases and a CRL for Amphibian Diseases need to be appointed by the EC.

When we take the WP2 list and the lists of 2006/88/EC together, for the exotic diseases, there is expertise on these diseases/pathogens mostly outside Europe, sometimes in the OIE. To be prepared for diagnosis of suspicion of one of these diseases/pathogens, it is necessary to acquire knowledge on their diagnosis in Europe. This means the EC needs to acquire expertise on the exotic diseases, and needs to fund the organization of training on techniques by CRL’s for NRL’s. This is followed by implementation of tests at NRL level, and their standardization and validation at each individual laboratory, funded by the national government.

2.2 Recommendations to achieve harmonized implementation
The task force of WP4 has made the following recommendations for guidelines and policy/legislation to achieve the aim:

For current EC listed exotic hazards, like Infectious Salmon Anaemia (ISA) there is already much knowledge at the CRL and NRL’s in Europe. Only for those laboratories, which have recently accessed the EC, workshops could be organized, to acquire knowledge and technical skills.

For EC non-exotic diseases/pathogens and non-exotic hazards identified by WP2, there is already much knowledge at the CRL and NRL’s in Europe. Workshops could be organized for labs, which need it, to acquire knowledge and technical skills.
For new exotic hazards (diseases/pathogens) from the exotic disease list of 2006/88/EC and from the WP2 list, there is very little or no knowledge yet within Europe: Therefore it is necessary, to first build capacity and training, then implementation, then harmonisation (with funding) through training again:

- The EC will appoint CRL’s for Crustacean diseases and Amphibian diseases, according to 2006/88/EC
- World wide specialists should be selected from the specific literature per disease/pathogen, as presented in the report of Deliverable 8 of PANDA
- Each CRL should have a leading or coordinating function for notifiable and emerging EC or WP2 listed diseases/pathogens
- Selected world wide specialists should be invited by the CRL’s, or specialists from the CRL’s should visit these specialists to acquire knowledge on the exotic diseases/pathogens
- Then specialists from the (PANDA) network should be identified, invited and funded: They are proposed to form ad hoc working group on those pathogens, coordinated by the CRL
- Funding of such actions will be essential for success, as all scientists already have projects of their institutes, and are too busy to do this additional work in spare time
- The ad hoc working groups make a plan for harmonisation and potential risk mitigation in the EC. Thereby, the cost-benefit of implementation will be important
- Each CRL should also identify specialists for the non-(OIE/EU) listed other? WP2 diseases individually?.
- These specialists should be funded to be a representative within the EC, to implement diagnosis of these WP2 diseases/pathogens, and be ready to diagnose the disease if suspicion occurs within the EC. As example there are various fish parasites listed in WP2
- The CRL could send a yearly small questionnaire to all NRL’s (per target group of aquatic animals) on gaps in knowledge, and training needs on screening and confirmative diagnostic tests of the EC/WP2 listed diseases. The results would then be discussed during each Annual meeting.
- Each CRL should coordinate the preparation of disease diagnosis leaflets, which are informative on the EC/OIE/WP2 listed diseases on diagnosis, and their standardisation and reference laboratories. These leaflets should be open accessible at the CRL and NRL websites, and a hard copy distributed to all NRL’s and regional European specialized laboratories (depending on the target group of aquaculture animals), the PANDA and EAFP members, and other interested specialists in the field. The leaflets and their distribution should be paid by the EC.
- These disease diagnosis leaflets could cover the following fields:
  - Name of disease and pathogen (and year of publication)
  - Description of disease (including pictures of clinical signs)
  - Susceptible animal species
  - Description of pathogen
  - Confirmative techniques for the disease
  - Screening techniques for the pathogen
  - Comments on available techniques (including QA status, costs, gaps)
  - Ring tests available? Who organizes them for whom?
  - EU-listed: yes/no
  - OIE-listed: yes/no
  - Reference laboratory (and expert with E-mail address, website)
  - Literature
- The EC should make production, publication and distribution of the *disease diagnosis leaflets* possible, via coordination with the CRL’s
- The EC could coordinate the education by direct contact with the CRL’s, and participation in the Annual meetings of CRL & NRL’s.
- It is important to use the right sampling procedure for new diseases/pathogens. This is not covered by WP4, but is an aspect of implementation of the new Directive 2006/88/EC. The NRL’s would have an important task in this, educating their field vets in sampling procedures.

Extension of tasks of the CRL’s is theoretically fine, and could be done in the new EC directive 2006/88/EC, but could give problems in reality. The number of diseases which should be covered by each CRL could go far over their limit. It would mean tasks would need to be divided over more laboratories. Which other laboratories would be relevant to support the CRL function is not determined by the task force of PANDA. This needs a political discussion at EC-level, whereby the CRL can propose certain laboratories to be candidate for that support function. An independent ad hoc group of experts of the EC could judge the proposal, and appoint other laboratories accordingly.

In Figure 1, the proposed organization to achieve this PANDA-goal is visualized:
Figure 2.2.a: Proposed organization to achieve harmonized implementation of confirmation and screening methods throughout Europe:

1) funding
2) responsibility and appointment
3) send yearly questionnaire on diagnostic methods
4) organize Annual meeting
5) ring test
6) provide biologics and standard operating procedures for tests
7) organize lab training workshops
8) provide data on test results, gaps in knowledge/diagnosis
9) organize training on sampling methods and diagnosis
10) invitation of experts & funding of Annual meetings
11) recruitment of experts for advisory panels
12) exchange of information/legislation
13) send diagnostic materials to the lab
14) make plans for harmonisation and potential risk mitigation in the EC

N.B. OIE = Office International des Epizooties, EC = European Commission, EFSA = European Food Safety Authority, CRL = Community Reference Laboratory, NRL = National Reference Laboratory.
2.3 Gaps in knowledge, training etc.

In the report D8 (Deliverable 8), Table 5.1a and b. present, summarize current screening/diagnostic methods and their evaluation. From that table, the knowledge and training needs are extracted and presented in Table 2. The task force of WP4 identified 5 additional important diseases/pathogens of molluscs or crustaceans, and evaluated them in Annex 8.5 of the D8 report. From that data, Table 3 of this report has been extracted.

Table 2.3.a: Gaps in knowledge, training etc., of WP2-listed diseases/pathogens, identified in WP4:

<table>
<thead>
<tr>
<th>Disease/Pathogen</th>
<th>Evaluation</th>
</tr>
</thead>
</table>
| **EHNV**                      | • Many good tests for screening and confirmation  
• RANA-project has organized a ring test.  
• Diagnosis of EHNV is not yet established at NRL’s:  
• advised to extrapolate ring test to NRL’s of EU, because of listed EHNV in 2006/88/EC : training needed.  
• PCR is now validated in Finland.                                                                 |
| **RSIV**                      | • Useful tests for screening and confirmation.  
• RSIV is not listed or tested in the EU yet  
• Cell culture (BF-2 a.o.) can be used to isolate the virus  
• Implementation of confirmative tests needed in Europe, via CRL Annual Meetings.                                                                 |
| **ISAV**                      | • The disease and pathogen are well documented in literature  
• Many good tests exist for screening and confirmation  
• There are no training needs.                                                                                                                                 |
| **KHV**                       | • Many good tests exist ?.  
• PCR ring test is organized by the OIE ref lab (CEFAS)  
• Tests get more sensitive, but latent carriers of KHV possibly cannot be detected yet.  
• Sequence of the marker vaccine is secret→PCR positive results of field strains cannot be distinguished from those of the vaccine strain of KHV.  
• The (TaqMan) PCR is the test of choice, to be validated by the ring test.  
• There are training needs on KHV detection and diagnosis, especially in Eastern Europe.                                                                                                                                 |
| *Streptococcus agalactiae*    | • Useful tests for identification, but time consuming  
• Disease problems with this pathogen increase→fast and accurate tests needed.  
• 16S RNA typing is important: needs validation, which means ring testing. Which lab is going to take this task is not defined yet. |
| *Streptococcus iniae*         | • Useful tests for identification, but time consuming  
• Disease problems with this pathogen increase→fast and accurate tests needed.  
• 16S RNA typing is important: needs validation, which means ring testing. Which lab is going to take this task is not defined yet. |
<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Description</th>
</tr>
</thead>
</table>
| *Lactococcus garviae*          | - Useful tests for identification, but time consuming  
- Disease problems with this pathogen increase → fast and accurate tests needed.  
- 16S RNA typing is important: needs validation, which means ring testing. Which lab is going to take this task is not defined yet. |
| *Trypanosoma salmositica*     | - Little experience with this pathogen in Europe  
- Very few specialists around the world.  
- Molecular biological methods for this parasite lack.  
- Training is needed, in clinics, detection methods and confirmative methods. Which lab takes the lead in the EC? |
| *Ceratomyxa shasta*            | - Little experience with this pathogen in Europe  
- Very few specialists around the world.  
- Molecular biological methods for this parasite lack.  
- Training is needed, in clinics, detection methods and confirmative methods. Which lab takes the lead in the EC? |
| *Neoparamoeba pemaquidensis*   | - Little experience with this pathogen in Europe  
- Very few specialists around the world.  
- Molecular biological methods for this parasite lack.  
- Training is needed, in clinics, detection methods and confirmative methods. Which lab takes the lead in the EC? |
| *Parvicapsula pseudobranchi-cola* | - Only experience in Norway with this salmon pathogen  
- Very few specialists around the world.  
- Although there is a PCR, it should be validated by other methods, which lack.  
- Training is needed, in clinics, detection methods and confirmative methods. Which lab takes the lead in the EC? |
| *Gyrodactylus salaris*         | - Good tests available  
- Diagnostic workshop was there for all NRL’s of the EC  
- Possibly interest in the later accessed EU-members states of especially Eastern Europe to do a diagnostic training related to this parasite. |
| *Aphanomyces invadans*         | - This fungus causes disease with very specific clinics  
- That makes a possible suspicion very doubtful.  
- Only 1 lab in Europe specialized (CEFAS).  
- From May 2008 all NRL’s should be able to diagnose EUS: urgently training needed in clinical pathology and diagnosis. |
| Mollusc diseases               | - The NRL network with the CRL keeps close contact on the available diagnostic methods on mollusc disease diagnosis.  
- Especially histopathology training for new pathogens or diseases is needed and organized by the CRL, who looks after the quality of diagnosis at NRL’s through ring tests and the Annual NRL meeting and workshops. |
| Crustacean Yellow head         | - Good tests available internationally  
- Most EU countries are not yet familiar with them  
- There are no CRL-NRL meetings on crustacean diseases yet.  
- As the disease is listed in 2006/88/EC, urgently training is needed in detection and diagnostic methods.  
- A CRL will be appointed soon by the EU, and will need to train the |
<table>
<thead>
<tr>
<th><strong>NRL’s for crustacean diseases.</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Crustacean White spot</strong></td>
</tr>
<tr>
<td>- good tests available internationally</td>
</tr>
<tr>
<td>- most EU countries are not yet familiar with them</td>
</tr>
<tr>
<td>- There are no CRL-NRL meetings on crustacean diseases yet.</td>
</tr>
<tr>
<td>- As the disease is listed in 2006/88/EC, urgently training is needed in detection and diagnostic methods.</td>
</tr>
<tr>
<td>- A CRL will be appointed soon by the EU, and will need to train the NRL’s for crustacean diseases.</td>
</tr>
<tr>
<td><strong>Crustacean Taura</strong></td>
</tr>
<tr>
<td>- good tests available internationally</td>
</tr>
<tr>
<td>- most EU countries are not yet familiar with them</td>
</tr>
<tr>
<td>- There are no CRL-NRL meetings on crustacean diseases yet.</td>
</tr>
<tr>
<td>- As the disease is listed in 2006/88/EC, urgently training is needed in detection and diagnostic methods.</td>
</tr>
<tr>
<td>- A CRL will be appointed soon by the EU, and will need to train the NRL’s for crustacean diseases.</td>
</tr>
<tr>
<td><strong>Crustacean IHHNV</strong></td>
</tr>
<tr>
<td>- good tests available internationally</td>
</tr>
<tr>
<td>- most EU countries are not yet familiar with them</td>
</tr>
<tr>
<td>- There are no CRL-NRL meetings on crustacean diseases yet.</td>
</tr>
<tr>
<td>- As the disease is listed in 2006/88/EC, urgently training is needed in detection and diagnostic methods.</td>
</tr>
<tr>
<td>- A CRL will be appointed soon by the EU, and will need to train the NRL’s for crustacean diseases.</td>
</tr>
<tr>
<td><strong>Coxiella cheraxi</strong></td>
</tr>
<tr>
<td>- No specialists present in Europe</td>
</tr>
<tr>
<td>- Training needed, but no specific tests are available</td>
</tr>
<tr>
<td><strong>Amphibian Irido-viridae Rana virus</strong></td>
</tr>
<tr>
<td>- only diagnosed at 1 or 2 labs in Europe</td>
</tr>
<tr>
<td>- Urgently training is needed: the RANA-project outcome should be extrapolated (ring test e.g.), and training in diagnosing these viruses should be parallel to that of EHNV.</td>
</tr>
<tr>
<td><strong>Amphibian Batrachochytrium dendrobatidis</strong></td>
</tr>
<tr>
<td>- There is no known lab in Europe yet diagnosing it.</td>
</tr>
<tr>
<td>- As the disease is emerging, there should be at least one national lab to be trained to diagnose the disease: clinics, isolation, and testing for confirmation.</td>
</tr>
</tbody>
</table>
Table 2.3.b: Gaps in knowledge, training etc., of some important non-WP2-listed diseases/pathogens, identified in WP4:

<table>
<thead>
<tr>
<th>Disease/pathogen</th>
<th>Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>OsHV-1</td>
<td>• No special further test needs</td>
</tr>
<tr>
<td></td>
<td>• Apart from NRL meeting no training needs</td>
</tr>
<tr>
<td>Bonamia ostreae</td>
<td>• Apart from NRL meeting no training needs</td>
</tr>
<tr>
<td>Marteilia refringens</td>
<td>• Apart from NRL meeting no training needs</td>
</tr>
<tr>
<td>Gaffkemia Aerococcus viridans</td>
<td>• Methods are o.k.</td>
</tr>
<tr>
<td></td>
<td>• No training needed</td>
</tr>
<tr>
<td>Crayfish plague Aphanomyces astaci</td>
<td>• Pathogen with high impact to Europe</td>
</tr>
<tr>
<td></td>
<td>• Urgently training needed on clinics, and detection and diagnostic methods</td>
</tr>
</tbody>
</table>
3. CONCLUSIONS

- Many good tests for screening and confirmation exist, at least well established in practice
- Validation is needed for many tests, at individual laboratories
- CRL’s for crustacean and amphibian diseases need urgently to be appointed
- The newly EC listed diseases of especially fish and crustaceans need urgent expertise acquirement and training at EC level
- Additionally, crayfish plague, caused by *Aphanomyces astaci* needs attention for diagnosis at EC level
- For the exotic diseases/pathogens this knowledge is to be extracted from outside Europe, via invitation of experts or working visits to their lab, by the CRL
- As the amphibian pathogens RANA virus and *Batrachochytrium dendrobatidis* are new to most laboratories, appointment of a CRL by the EC is recommended, after which certain laboratories should get expertise and skills in testing via training from this CRL.
- According to the task force of WP4 of PANDA:
  - The EC needs to take responsibility in funding the process of acquiring knowledge and skills, and communication (leaflets) at CRL level
  - The CRL functions will expand, and possible division of tasks to support labs is suggested, and ad hoc expert groups to plan the process
  - The NRL functions will also expand, but to a limited extent
  - The NRL’s or regional labs should organize training on sampling methods and diagnosis for field vets among others.
- The PANDA network will be further consulted for this aim.
4. ACKNOWLEDGEMENTS

The PANDA project was funded by Directorate General Research of the European Commission. We thank them for their support, which made this project possible, including the travels and strengthening of our international networks.

The task force members of WP4 are thanked for their valuable input and time, to the PANDA project, and especially to deliverables 8 and 9.