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RESULTS OF THE PROFICIENCY TEST, PT1 AND PT2, 2012

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Abstract:

A comparative test of diagnostic procedures was provided by the European Union Reference Laboratory (EURL) for Fish Diseases. The test was divided into proficiency test 1 (PT1) and proficiency test 2 (PT2).
The number of National Reference Laboratories (NRLs) participating in PT1 and PT2 was 43.
The tests were sent from the EURL in the beginning of September 2012.
Both PT1 and PT2 are accredited by DANAK under registration number 515 for proficiency testing according to the quality assurance standard DS/EN ISO/IEC 17043.

PT1 consisted of five coded ampoules (I-V). These ampoules contained IPNV, EHNV, SVCV, IHNV and VHSV, respectively, see table 1. The proficiency test was designed to primarily assess the ability of participating laboratories to identify the listed fish viruses VHSV, IHNV and ENHV (Council Directive 2006/88/EC) and the non-listed viruses SVCV and IPNV if present in the ampoules, bearing in mind that the test ampoules also could contain other viruses (e.g. other fish rhabdoviruses, ranaviruses, or birnaviruses). In addition the participants were asked to quantify the viruses in PT1 by titration in order to assess the susceptibility of their fish cell lines for virus infection. Participants were encouraged to use their normal standard laboratory procedures. However, the identification should be performed according to the procedures laid down in Commission Decision 2001/183/EC using fish cell cultures followed by e.g. ELISA, PCR, immunofluorescence (IFAT) or neutralisation test.
If ranavirus was present in any of the ampoules, it was mandatory to perform sequence analysis or a restriction endonuclease analysis (REA) of the isolate in order to determine if the isolate was EHNV or another ranavirus and it was recommended to follow the procedures described in Chapter 2.3.1 in the OIE Manual of Diagnostic Tests for Aquatic Animals 2009. Laboratories were encouraged to identify VHSV and IHNV isolates as far as possible by means of genotyping in addition to the standard methods. It was recommended to use the genotype notification described in Einer-Jensen et *al*. (2004) for VHSV and in Kurath et *al*. (2003) for IHNV. Laboratories were encouraged to submit all sequencing results used for genotyping the isolates.

PT1 Conclusion

The inter-laboratory proficiency test 2012 was conducted without major constraints. 92% of parcels were delivered by the shipping companies within 8 days after submission. It was, however, unfortunate that two parcels were 20 days on the way and one parcel was 43 days on the way before delivered to the laboratory primarily due to border controls. Two parcels never left the EURL. In one case this was dued to delivery restriction for such reagents (Iran), in the other case because the fetal bovine serum (FBS) used was from a country not certified free from foot and mouth disease.
In the meantime the batch of serum currently used in the EURL for cell culture has tested negative for foot and mouth disease virus (FMDV) following accredited procedures.
In 2009 EHNV was included in the proficiency test for the first time this year 36 participants were able to correctly identify the virus. Of the laboratories performing PCR based methods, 33 laboratories performed sequencing. Of these laboratories all correctly identified the content. Two laboratories performed REA and one laboratory performed restriction enzyme fragmentation.

PT2
consisted of four coded ampoules (VI-IX). The ampoules contained ISAV and KHV. Furthermore, one ampoule contained *Aphanomyces invadans* and one sterile pyrogen free water, see table 11. The test was designed to primarily assess the ability of participating laboratories to identify the notifiable fish pathogens ISAV, KHV and *A. invadans* (listed in Council Directive 2006/88/EC) if present in the ampoules, bearing in mind that the test ampoules could also contain other pathogens. Participants were expected to use their normal PCR or real-time PCR methods for detection of the pathogens. It was not mandatory to grow KHV and ISAV on cell cultures in this test, but the viruses were not inactivated and, thus, it might had been possible to replicate them in cell cultures.
If present, only **inactivated** *A. invadans* was included in the ampoules.

PT2 conclusion
Considering that this was the third time that the EURL provided a proficiency test on ISAV and KHV identification, and the second time that the EURL provided a proficiency test on *A. invadans*, we consider that most participants obtained satisfying results. Out of 34 laboratories testing for *A. invadans* all 34 identified the pathogen in ampoule VI. Out of 38 laboratories performing KHV identification, 36 laboratories identified KHV in ampoule VII. Out of 39 laboratories 32 laboratories identified Not *A.invadans, KHV or* ISAV in ampoule VIII. Out of 38 laboratories performing ISAV identification 36 identified ISAV in ampoule IX. Very significant improvement in the proficiency of identifying and typing these pathogens has been observed during these 3 years. In autumn 2012 the European Commission decided to de-list EUS and it is officially no more considered as an exotic disease in the Union. However we find that a certain level of preparedness for the introduction of this disease in European aquaculture should be maintained. But it is still unclear whether the pathogen will be included in future inter-laboratory proficiency tests or not and the topic will be discussed at our next Annual Meeting in May 2013.
It is an appreciated matter of fact that many laboratories are putting efforts in performing genetic characterizing the isolates through sequence analysis as it is important that laboratories can discriminate between isolates as e.g. pathogenic ISAV strains with deletions in the HPR region and HPR0 strains.

The results of the proficiency tests will be further discussed at this presentation.