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RESULTS OF THE PROFICIENCY TEST, PT1 AND PT2, 2011

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A comparative test of diagnostic procedures was provided by the EU Reference Laboratory (EURL) for Fish Diseases to 41 National Reference Laboratories (NRLs) in the start of middle of October 2011. The test was prepared and tested according to protocols accredited by DANAK under registration number 515 to proficiency testing according to the quality assurance standard DS/EN ISO/IEC 17043. The test consisted of 2 tests: PT1 and PT2.

PT1 Introduction

PT1 consisted of five coded ampoules (I-V). The ampoules contained VHSV, EHNV, European catfish virus (ECV), IHNV+IPNV and IPNV, respectively. The proficiency test was designed to primarily assess the ability of participating laboratories to identify the fish viruses VHSV, IHNV and ENHV (all listed in Council Directive 2006/88/EC) 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. neutralisation test, immunofluorescence, ELISA or PCR. If ranavirus was present in any of the ampoules, it was mandatory to perform a 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

All laboratories identified VHSV without problems. As IHNV was included as a double infection with IPNV some laboratories failed to correctly identify this virus. IPNV was correctly identified by 36 of the 41 laboratories. In 2009 EHNV was included in the proficiency test for the first time and 32 participants were able to correctly identify the virus. This year EHNV was included as well as ECV, both belong to the ranavirus family. Of the laboratories performing PCR based methods, 31 laboratories performed sequencing for ampoule II and 32 for ampoule III. Of these laboratories all correctly identified the content in ampoule II as EHNV and 31 correctly identified the content in ampoule III as ECV/ESV. One laboratory performed both sequencing and REA for both ampoule II and III without being able to identify which type of ranavirus the isolates belong to. One laboratory performed REA only for both ampoule II and III and was able to identify the isolate as either EHNV or ranavirus, not EHNV.

All titres submitted by participants for each cell line and ampoule, respectively were compared to each other. In this way, the titres obtained by each laboratory were plotted in relation to the combined submitted data set and each participating laboratory should be able to compare the sensitivity of their cell lines to the sensitivity of those used by the other participating laboratories. We recommend all participants to evaluate the sensitivity of the cells used in their laboratory in relation to the diagnostic purpose.

PT2 Introduction

PT2 also consisted of five coded ampoules (VI-X). The ampoules contained ISAV and KHV. Furthermore, one ampoule contained *Aphanomyces invadans*. It was decided at the 15th Annual Meeting of the NRLs for Fish Diseases in Aarhus 26-27 May 2011, that testing for *A. invadans* for the first time should be included in the yearly proficiency test provided by the EURL. The test was designed to primarily assess the ability of participating laboratories to identify the notifiable fish pathogens ISAV, KHV and *A. invadans* if present in the ampoules, bearing in mind that the test ampoules could also contain other viruses. 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 they should thus be possible to amplify in cell cultures. If present, only **inactivated** *A. invadans* was included in the ampoules.

PT2 conclusion

Considering that this was the second time that the EURL provided a proficiency test on ISAV and KHV identification, and the first time that the EURL provided a proficiency test on *A. invadans*, we consider that most participants obtained satisfying results. Out of 36 laboratories performing ISAV identification 32 identified ISAV in ampoule VI containing low titre ISAV and 35 identified ISAV in ampoule VII containing high titre ISAV. All 37 laboratories testing for KHV identified KHV in ampoule VIII containing high titre KHV, and 36 of them identified KHV in ampoule X containing low titre KHV. Out of 31 laboratories testing for *A. invadans* 28 identified the pathogen in ampoule IX.

Lowering the titre of the virus caused only one laboratory to miss identification of KHV in the low titered ampoule X. A reason for the laboratory to miss the correct identification is most likely due to mistaken marking of the ampoule. If this is the reason then all laboratories testing for KHV were able to identify both the high titre and the low titre KHV. For ISAV, one laboratory missed identification in the high titre ampoule and for the low titre ampoule three further laboratories did not succeed in the identification.

A couple of laboratories identified pathogens not present in the ampoules. E.g. one laboratory identified ISAV in all ampoules but only with a weak positive reaction in the ampoules where ISAV was not present.

A critical point in PCR based diagnostic tools is avoiding false positive and false negative results. To decrease the risk of having false negative results, it is always recommended that laboratories use the most sensitive tool available, validate the sensitivity of their diagnostic tools and use proper controls. To decrease the risk of false positive results laboratories have to be very aware of the risk of cross contaminations.

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