Part I: Summary of general activities related to the disease

1. Test(s) in use/or available for the specified disease/topic at your laboratory

Evira applies the following diagnostic methods for the detection and analysis of crayfish plague *Aphanomyces astaci:*

Culture method: An own modification of the method of Cerenius et al 1988 (Viljamaa-Dirks and Heinikainen 2006). The test is suitable for isolation of the agent from diseased, preferably moribund or freshly dead animals.

Molecular tests:

- A PCR method based on detection of DNA in ITS regions of the 5.8 rRNA gene, described by Oidtmann et al in 2006, is used mainly to verify the isolated oomycetes as *A. astaci*

- A TaqMan MGB real time PCR method, described by Vrålstad et al in 2009, is used to detect crayfish plague from the exoskeleton of diseased or carrier crayfish.

- A randomly amplified polymorphic DNA PCR method (RAPD-PCR), described by Huang et al in 1994, is used to examine the genetic group of isolates of *A. astaci*

Investigations in 2010:

Test	***For***	***number of animals/cases investigated***	***positive cases***
culture	isolation of the pathogen	46/16	9
real time PCR	presence of the pathogen in native crayfish	appr 500/37	16
real time PCR	presence of the pathogen in non-native (North-American) crayfish	7/4	3

2. Production and distribution of diagnostic reagents

In the end of the year 2010 there were 104 viable strains in the *Aphanomyces astaci* strain collection.

Type of reagent	***Amount supplied nationally***	***Amount supplied to other countries***
Reference strains	1	1
extracted DNA		1x

Part II: Activities specifically related to the mandate of OIE Reference Laboratories

3. International harmonisation and standardisation of methods for diagnostic testing or the production and testing of vaccines

A crayfish plague research consortium was formed in 2009 and has partners from Finland, Sweden, Estonia, France, Spain, Germany and Italy. One of the aims of this consortium is to develop standardised diagnostic methods for crayfish plague. Evira is the responsible partner of the sub-task of comparing the existing PCR-based methods for specificity and sensitivity. Funding for this consortium work has not been found yet.

4. Preparation and supply of international reference standards for diagnostic tests or vaccines

None

5. Research and development of new procedures for diagnosis and control

A PCR-based genotype-specific diagnostic method was developed (Heinikainen and Viljamaa-Dirks 2010), which differentiates the genotypes As and PsI. Epidemiological studies of these two genotypes are now possible even when the isolation process to gain a pure culture fails, or is not possible due to the quality of samples. This method is still in validation process and further developed to improve the sensitivity to be sufficient for detection also in the symptom free carrier crayfish.

6. Collection, analysis and dissemination of epizootiological data relevant to international disease control

In connection with a slowly progressing epizootic in a large river in Lapland, samples have been collected from the affected river during the last four years and studied with real time PCR method. In 2010, majority of the sampled crayfish (51/55) from a location recognised as infected in 2007, showed no signs of infection and gave negative results in PCR. Because of the possible existence of low-virulent strains in other Finnish noble crayfish populations, also other water bodies are screened for crayfish and crayfish plague. A wider survey was started in co-operation with the Finnish Forest and Park Service and the Finnish Game and Fisheries Institute.

The disease situation concerning crayfish plague is poorly known in most of the member countries, and mostly not reported to the veterinary authorities. It seems that there is often lack of information between fisheries and veterinary authorities concerning the status of crayfish plague, since the infection is listed as a notifiable animal disease in only a few member countries. In lack of information countries tend to report the status as not infected, even when general knowledge claims the contrary. This situation is hopefully improving in the future, reliable diagnostic methods now being more easily available.

One sample of spiny cheek crayfish (*Orconectes limosus*) was received from Czech Republic. Isolates from these crayfish, verified as *Aphanomyces astaci* by PCR and infection trials, do not seem to belong to the genotypes described so far. A case report is in preparation, as well as further characterisation of these isolates.

7. Provision of consultant expertise to OIE or to OIE Members

None in 2010

8. Provision of scientific and technical training to personnel from other OIE Members

One PhD student from Norway (Norwegian Veterinary Institute) was trained in isolation method of crayfish plague.

9. Provision of diagnostic testing facilities to other OIE Members

Crayfish samples from Estonia from two locations were received, and one location was judged positive for crayfish plague by real-time PCR method. This finding was reported to the official OIE delegate of Estonia for further report to OIE.

The OIE delegate of Czech Republic was informed about the positive results of the examination of the spiny cheek crayfish.

10. Organisation of international scientific meetings on behalf of OIE or other international bodies

None in 2010.

11. Participation in international scientific collaborative studies

Evira participates in a Norwegian research project "Monitoring for crayfish plague" co-ordinated by the Norwegian Veterinary Institute. The main task of this project is to study the possibilities to monitor the crayfish plague spores directly from the environment with PCR. In connection with this project, a Norwegian project researcher stayed in Kuopio for the summer months, collecting field samples of crayfish plague outbreaks and doing experimental work in Evira as well as in the University of Eastern Finland. This project is still on-going.

12. Publication and dissemination of information relevant to the work of OIE (including list of scientific publications, internet publishing activities, presentations at international conferences)

- ***Presentations at international conferences and meetings***

A poster and oral presentation was given in the Autumn School in biodiversity of Saprolegnia in Madrid, Spain, concerning the development of the genotype specific diagnostic PCR-method.

- ***Scientific publications in peer-reviewed journals***

None in 2010.

- ***Other communications***

None in 2010.

13. Inscription of diagnostic kits on the OIE Register

i) Did you participate in expert panels for the validation of candidate kits for inscription on the OIE Register? If yes, for which kits?

No

ii) Did you submit to the OIE candidate kits for inscription on the OIE Register? If yes, for which kits?

No.

References:

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