

OIE Reference Laboratory Reports

Activities in 2011

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 the 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.
- A PCR method based on detection of DNA in ITS regions of the 5.8 rRNA gene (Oidtmann et al 2006) is used mainly to verify the isolated oomycetes as *A. astaci*
- A TaqMan MGB real time PCR method (Vrålstad et al 2009) is used to detect crayfish plague from the exoskeleton of diseased or carrier crayfish.
- A randomly amplified polymorphic DNA PCR method (RAPD-PCR)(Huang et al 1994) is used to examine the genetic group of the isolates of *A. astaci*
- a semi-nested PCR method for the differentiation of *A. astaci* genotypes Ps1 and As (Heinikainen,unpublished) is under study

Number of crayfish investigated in 2011:

Test	For	specificity	total
culture	isolation of the pathogen	<i>Aphanomyces</i> sp.	34
PCR Oidtmann 2006	identification of <i>A. astaci</i>	<i>Aphanomyces astaci</i>	9
real time PCR	presence of the pathogen in crayfish exoskeleton	<i>Aphanomyces astaci</i>	684
RAPD-PCR	identification of the genotype (culture)	genotypes Ps1,Ps2,As,Pc	9
semi-nested PCR Heinikainen	identification of the genotypes (from exoskeleton)	Ps1,As	1

2. Production and distribution of diagnostic reagents

In the end of the year 2011 there were 113 viable strains in the *Aphanomyces astaci* strain collection.

Type of reagent	Amount supplied nationally	Amount supplied to other countries
Reference strains	none	4
extracted DNA	none	1
infected signal crayfish in ethanol	none	1

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) Establishment and maintenance of a network with other OIE Reference Laboratories designated for the same pathogen or disease and organisation of regular inter-laboratory proficiency testing to ensure comparability of results

- International ring trials has not been organised yet, but the initiative has been discussed with the other OIE expert for crayfish plague, Birgit Oidtmann from CEFAS.
- It was agreed with CEFAS that an external quality control could be received (based on PCR) to verify a positive isolation when applying the culture method.

b) Organisation of inter-laboratory proficiency testing with laboratories other than OIE Reference Laboratories for the same pathogens and diseases to ensure equivalence of results

- Interlaboratory test evaluation was started with the Norwegian Veterinary Institute by sharing a batch of 50 exoskeleton samples for real time PCR method. The results are pending.

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

We can supply reference strains of *Aphanomyces astaci*, as well as DNA extractions of pure cultures or infected crayfish exoskeleton. We have also provided infected crayfish (*Pacifastacus leniusculus*) to a Czech university.

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

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 (Strand et al. 2011).

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

In connection with a slowly progressing epizootic affecting noble crayfish in a large river in Lapland, samples have been collected during the last five years and studied with real time PCR method. The infection is slowly spreading upwards, leaving the downstream part of the river unproductive. A plague prevalence of about 13% can be confirmed in affected but still catchable populations according to the samples received in 2010 and 2011.

Because of the possible existence of low-virulent strains in other Finnish noble crayfish populations, also other water bodies were screened for crayfish and crayfish plague in a wider survey in co-operation with the Finnish Forest and Park Service and the Finnish Game and Fisheries Institute. Altogether 530 crayfish were collected, from eight localities. The results of this survey are pending.

One sample of spiny cheek crayfish (*Orconectes limosus*) was received from Czech Republic in 2010. Isolates from these crayfish, verified as *Aphanomyces astaci* by PCR and infection trials, did not belong to the genotypes described so far. A case report was published (in 13.:Kozubíková et al 2011), and a further characterisation of these isolates was started.

7. Maintenance of a system of quality assurance, biosafety and biosecurity relevant to the pathogen and the disease concerned

The laboratory is qualified according to the ISO 17025 standard by The Finnish Accreditation Service. The crayfish plague culture method is accredited and the PCR method is accredited for a flexible scope by the same institute.

8. Provision of consultant expertise to OIE or to OIE Member Countries

Chapter for the crayfish plague in the OIE Aquatic Manual 2012 was reviewed. Photo material from crayfish plague infected crayfish and crayfish plague cultures was provided to a Japanese university for education purposes.

9. Provision of scientific and technical training to personnel from other OIE Member Countries

None in 2011.

10. Provision of diagnostic testing facilities to other OIE Member Countries

None in 2011.

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

None in 2011.

12. Participation in international scientific collaborative studies

Evira participates in a Norwegian research project “Monitoring for crayfish plague” co-ordinated by the Norwegian Veterinary Institute. Part of the experimental work was done in Evira aquarium facilities in 2010. The aim of those studies was to verify the temporal spore release from infected signal crayfish. The manuscript is in preparation.

13. 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*

Viljamaa-Dirks S, Heinikainen S, Lehvola H, Kozubikova E. A new genotype of the crayfish plague agent *Aphanomyces astaci* detected from the spiny-cheek crayfish *Orconectes limosus*. 15. EAFP Conference Diseases of Fish and Shellfish, Split, Croatia, 12.-16.9.2011. Poster P116, abstract book p. 290

■ *Scientific publications in peer-reviewed journals*

Viljamaa-Dirks S, Heinikainen S, Nieminen M, Vennerström P, Pelkonen S. 2011. Persistent infection by crayfish plague *Aphanomyces astaci* in a noble crayfish population – a case report. Bulletin of the European Association of Fish Pathologists 31(5): 182–188.

Kozubíková E, Viljamaa-Dirks S, Heinikainen S, Petrušek A. 2011. Spiny-cheek crayfish *Orconectes limosus* carry a novel genotype of the crayfish plague pathogen *Aphanomyces astaci*. Journal of Invertebrate Pathology 108:214-216.

14. Other communications

A web site for the OIE reference laboratory is maintained at

http://www.evira.fi/portal/en/evira/about_us/operation_areas/laboratory_operations/reference_laboratory_operations/international_operations/

References:

Cerenius, L., Söderhäll, K., Persson, M. and Ajaxon, R. 1988. The crayfish plague fungus, *Aphanomyces astaci* - diagnosis, isolation and pathobiology. *Freshwater crayfish* 7: 131-144.

Huang, T.-S., Cerenius, L. and Söderhäll, K. 1994. Analysis of genetic diversity in the crayfish plague fungus, *Aphanomyces astaci*, by random amplification of polymorphic DNA. *Aquaculture* 126: 1–9.

Oidtmann, B., Geiger, S., Steinbauer, P., Culas, A. and Hoffmann, R. 2006. Detection of *Aphanomyces astaci* in North-American crayfish by polymerase chain reaction. *Diseases of Aquatic Organisms* 72: 53–64.

Strand D., Holst-Jensen A., Viljugrein H., Edvardsen B., Klaveness D., Jussila J., Vrålstad T. 2011. Detection and quantification of the crayfish plague agent in natural waters: direct monitoring approach for aquatic environments. *Diseases of Aquatic Organisms* 95(1):9-17.

Viljamaa-Dirks, S. and Heinikainen, S. 2006. Improved detection of crayfish plague with a modified isolation method. *Freshwater Crayfish* 15:376–382.

Vrålstad, T., Knutsen, A., Tengs, T. and Holst-Jensen, A. 2009. A quantitative TaqMan® MGB real-time polymerase chain reaction based assay for detection of the causative agent of crayfish plague *Aphanomyces astaci*. *Veterinary microbiology* 137(1-2):146-155.