Activities in 2009

Crayfish plague

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Summary of general activities related to the disease

1. Test(s) in use/or available for the specified disease 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. The test is suitable for isolation of the agent from diseased, preferably moribund 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*

In 2009, about one hundred specimens of crayfish were studied by the cultivation method. About 30 isolates were tested by specific PCR and RAPD-PCR. Approximately 350 crayfish, including both noble and signal crayfish, were examined by the real-time PCR for the presence of crayfish plague.

2. Production and distribution of diagnostic reagents

Evira maintains a collection of crayfish plague *Aphanomyces astaci* strains, collected during the last ten years and consisting of strains of the two genotypes found in Finland, Astacus-type (As) and Pacifastacus-type (PsI). Culture method for isolation of crayfish plague is still applied in diagnostic work in order to collect fresh isolates of *A. astaci*. In the end of the year 2009 there were 85 viable strains in the collection. Purified DNA from both genotypes is prepared for diagnostic reference purposes. Nationally Evira is the only laboratory involved in laboratory diagnostics of crayfish plague, so crayfish plague DNA is supplied only for research purposes for agreed projects.

Purified DNA for diagnostic reference material was supplied to 4 European veterinary laboratories appointed as crustacean disease reference laboratories by the European Union. Examples of a low- and a high virulent strain were sent as culture to Austria for research purposes.

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

Evira is an active partner in a crayfish plague research consortium, which was formed in 2009 and has partners from Finland, Sweden, Estonia, France, Spain, Germany and Italy. The title of the research plan is Prevention of the spread of crayfish plague into and via aquaculture. One of the aims of this consortium is to develop a standardised diagnostic method for crayfish plague. First step is to compare the existing PCR-based methods for specificity and sensitivity. This work involves Evira, University of Eastern Finland, University of Landau-Koblenz and a commercial laboratory from Germany, Evira being the responsible partner of this sub-task. Funding has been applied from the European Union (EMIDA-Eranet).

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

International reference standards have not yet been developed for crayfish plague.

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

There is on-going research to develop a PCR-based genotype-specific diagnostic method, which would allow epidemiological studies of crayfish plague more easily than the existing culture based genotyping with RAPD-PCR. Research is done both in Evira and the University of Eastern Finland, based on the *A. astaci*- culture collection of Evira. This work has not yet proceeded to a validation process.

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

A very slow progress of a natural infection of noble crayfish *Astacus astacus* with an Astacus-genotype strain of crayfish plague was recorded in a river in Lapland, where the strain was isolated from the same, slowly declining population in a third successive year. Downstream populations have slowly disappeared, so it is apparent that the epizootic has not yet reached its maximum mortality in this stretch of the river. The longevity of the infection causes, however, extra challenge for the education of the fishery actors and general public. In connection of this epizootic, samples have been collected from the affected river during the last three years to judge the prevalence of the infection. The samples are studied with Real time PCR- method. This work is in progress. In connection 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 is planned in co-operation with the Finnish Forest and Park Service and the Finnish Wildlife 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, as shows the first report to OIE of the infection from Italy, the presumed origin of the European epidemic. As Evira has only acted a short period as a reference laboratory, contacts from other member countries have been limited. No samples have been received from other OIE-members that would make a notification to OIE necessary.

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

The chapter of crayfish plague for the Manual of Diagnostic Tests for Aquatic Animals, sixth edition, was reviewed.

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

One person from Estonia (Estonian University of Life Sciences) was trained in crayfish plague diagnostics including pathological study and PCR-based diagnostic methods during a two-week training period.

9. Provision of diagnostic testing facilities to other OIE Members

Crayfish samples from Estonia, consisting of samples from seven locations, were received but not through official veterinary services. Four locations were judged positive for crayfish plague by real-time PCR method. No reports were made to OIE or official veterinary services in Estonia, but the University of Life Sciences providing the samples was advised to report the cases to the veterinary authority for further report to OIE.

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

A small scale international seminar was organised in Kuopio, in August 2009 as a start for the activities as an OIE reference laboratory. This meeting was also attended by the other OIE crayfish plague expert dr. Birgit Oidtmann and the president of the OIE Aquatic Animals Commission, prof. Barry Hill. .

11. Participation in international scientific collaborative studies

In addition of the European project described in chapter 3, 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. 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 presentation was given in the bi-annual congress of the European Association of Fish Pathologists EAFP, held in Prague 14-19th September, concerning the exceptional features of the crayfish plague epidemic in Lapland ("Persistent *Aphanomyces astaci* infection in a large Northern noble crayfish river", Viljamaa-Dirks, Heinikainen, Muje, Pelkonen)

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

None in 2009

- ***Other communications***

A round-table discussion about crayfish diseases was attended in the Regional European Crayfish Workshop "Future of Native Crayfish in Europe" in Pisek, Czech Republic, 7-10th September.

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