
DTU

DIAGNOSTIC METHODS FOR THE SURVEILLANCE AND CONFIRMATION OF EPIZOOTIC HAEMATOPOIETIC NECROSIS

31st May 2021 – Shared session AW for national refrence laboratories for fish and crustacean diseases

CHAPTER 2.3.1.

INFECTION WITH EPIZOOTIC HAEMATOPOIETIC NECROSIS VIRUS

EPIZOOTIC HAEMATOPOIETIC NECROSIS LIST A(+D+E) 2018-1882

Exotic disease in EU.

Detection of the pathogen require immediate stamp out

Mass mortality of redfin perch. Photo: Anonymous

EHN LIST A (2018-1882)

Susceptible species

Redfin perch (perca fluviatilis) and rainbow trout

Vector species

Bighead carp (*Aristichthys nobilis*), goldfish (*Carassius auratus*), crucian carp (*Carassius carassius*), common carp and koi carp (*Cyprinus carpio*), silver carp (*Hypophthalmichthys molitrix*), chub (*Leuciscus* spp.), roach (*Rutilus rutilus*), rudd (*Scardinius erythrophthalmus*), tench (*Tinca tinca*)

OIE manual available

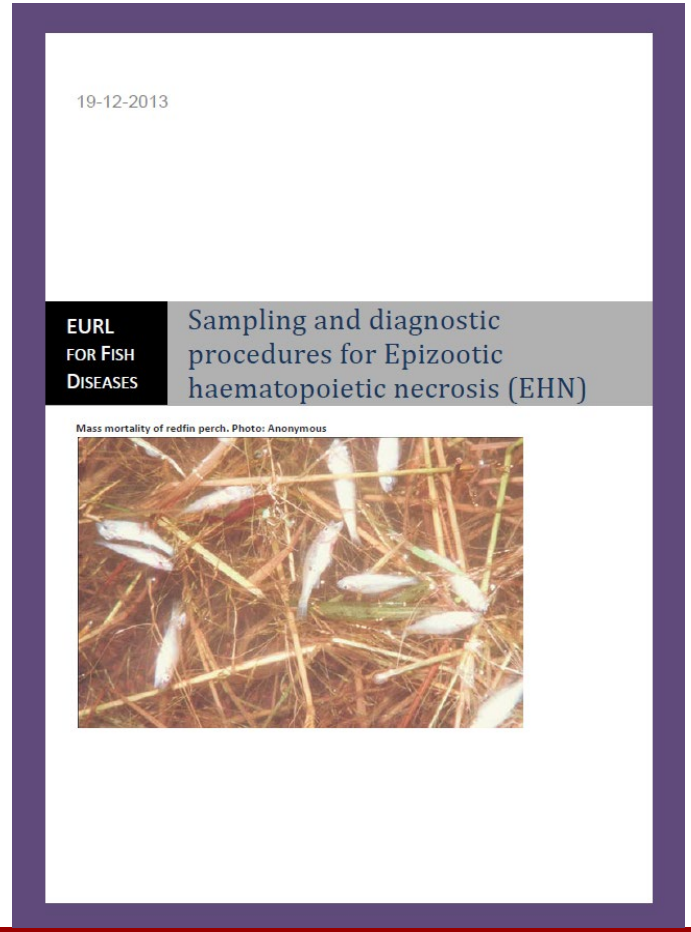
https://www.oie.int/fileadmin/Home/eng/Health_standards/aahm/current/chapitre_ehn.pdf

HOWEVER THERE IS AN ISSUE IN STRAIN IDENTIFICATION AND DISCRIMINATION BETWEEN EHNV AND OTHER RANAVIRUS

EHN

- We will revise what was provided by the EURL in 2014 – and included in 2015-1554 and upload a new manual for

- Sampling and diagnostic procedured for Epizootic Haematopoieti Necrosis EHN



For Surveillance - Detailed diagnostic methods and procedures for the surveillance of EHN

- To conduct surveillance:

- For the detection of EHNV, **kidney, spleen, heart and/or encephalon**;, **tissue material of up to 10 fish may be pooled**.

- FOR SURVEILLANCE For the surveillance of EHNV 2 options:
  – Cell culture based (as for infection with VHSV and IHNV)
- Or qPCR **shall** be used plus sequencing of MCP (OIE primers) for discrimination EHNV from other ranavirus

Primer/probe name	Primer/probe sequence	Melting temp. (°C)	Positions in MCP gene (5′ to 3′)	Amplicon size (nt) including primers
RanaMCPstdF	GTT CTC ACA CGC AGT CAA GG	53.8	891–910	359
RanaMCPstdR	CGG ACA GGG TGA CGT TAA G	53.2	1231–1249	
RanaF1	CCA GCC TGG TGT ACG AAA ACA	54.4	1040–1060	97
RanaR1	ACT GGG ATG GAG GTG GCA TA	53.8	1136–1117	
RanaP1	6FAM-TGG GAG TCG AGT ACT AC-MGB	47.1	1079–1095	

Sequencing

- The obtained clean consensus sequence shall be analyzed with BLASTX against the Reference proteins database (refseq_protein), matching (99 % identity) with a reference protein sequence of EHNV.

Primer name	Sequence	Cycling conditions	Product size
EHNV MCP-forward	5’-CGC-AGT-CAA-GGC-CTT-GAT-GT-3’	First round PCR 1 cycle: 94 °C 3 minutes	
EHNV MCP-reverse	5’-AAA-GAC-CCG-TTT-TGC-AGC-AAA-C-3’	35 cycles: 94 °C for 30 seconds 50 °C for 30 seconds 72 °C for 60 seconds 1 cycle: 72 °C for 5 minutes	580 bp

The relevance of Sequencing

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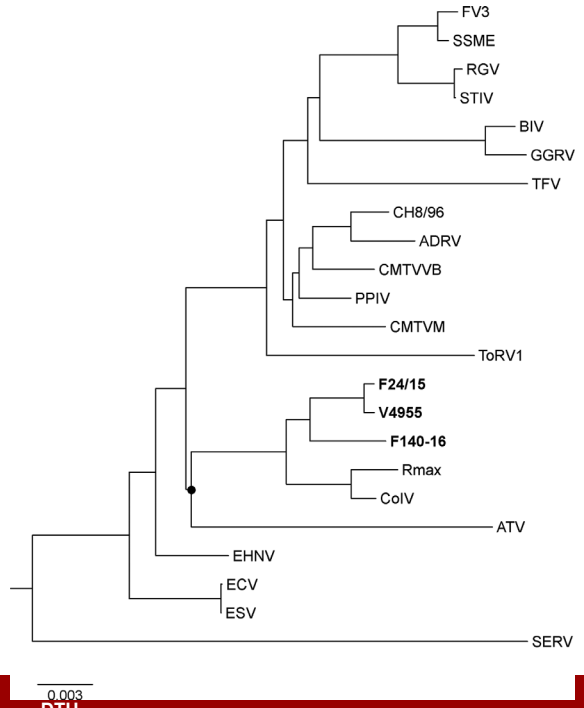
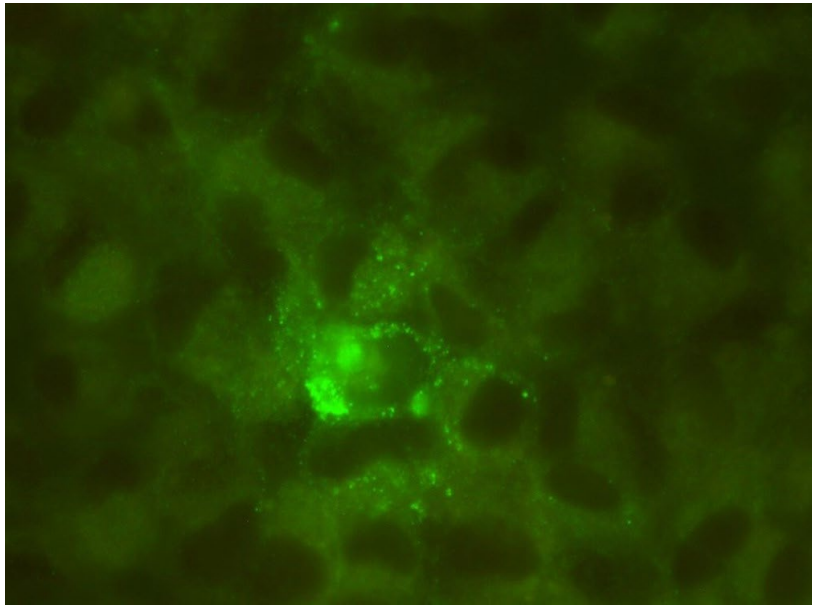
Characterization of ranaviruses isolated from lumpfish *Cyclopterus lumpus* L. in the North Atlantic area: proposal for a new ranavirus species (European North Atlantic Ranavirus)

Since 2014 detected a number of ranavirus (NOT EHNV) in Lumpfish in Scotland, Ireland, Faroe Island and Iceland.





For Diagnostics - Detailed diagnostic methods and procedures for the confirmation of the presence of or to rule out the suspicion of EHN

When a suspicion of EHN is required to be confirmed or ruled out, the following inspection, sampling and testing procedures shall be complied with:

(a) the farm under suspicion shall be subject to at least one health inspection and one sampling of 10 fish, when clinical signs or *post-mortem* signs consistent with infection with EHN are observed or minimum 30 fish, when clinical or *post-mortem* signs are not observed. Samples shall be tested using one or more of the diagnostic methods set out in points (i) and (ii) in accordance with the detailed diagnostic methods and procedures :

(i) conventional virus isolation in cell culture with subsequent molecular virus identification by sequencing;

(ii) virus detection by RT-qPCR with subsequent molecular virus identification by sequencing;

(b) the presence of EHN shall be considered as confirmed, if one or more of those diagnostic methods are positive for EHNV. The States, zones or compartments previously not infected shall be based on conventional virus isolation in cell culture with subsequent molecular virus identification or RT-qPCR with subsequent molecular virus identification; confirmation of the first case of EHN in Member