Facilities, services and challenges encountered in assisting WOAH Members

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WOAH Expert for Koi Herpesvirus (KHV) & Spring Viraemia of Carp (SVCV)

Establishment of a Regional Aquatic Animal Health Laboratory Network (RAAHLN) for Africa
5 - 7 December 2023

Cefas – who we are?

Executive agency of DEFRA responsible for the control of serious disease in aquatic animals in GB.

The Cefas hosts the UK government's Fish Health inspectorate (FHI) which has a legal duty to deliver the remit under the **Aquatic Animal Health (England and Wales) Regulation 2009**.

This is achieved this through:

- **Investigation and control of outbreaks of listed and emerging disease of aquatic animals, both on suspicion and on confirmation.**
- Investigation of unexplained or increased mortality in aquatic animals, both in aquaculture and in the wild.
- On site monitoring of aquatic animal health through inspection of aquaculture farms, processing and purification establishments, importers and dealers.

GB Listed diseases [ISO 17025 accredited assays]

Infection with *Gyrodactylus salaris*

Infectious Salmon Anaemia (ISA)

Viral haemorrhagic septicaemia (VHS)

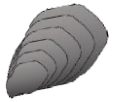
Infectious haematopoietic necrosis (IHN)

Epizootic haematopoietic necrosis (EHN)

Bacterial Kidney Disease (BKD)

Spring Viraemia of Carp (SVC)

Koi Herpesvirus (KHV)



Infection with *Bonomia exitiosa*

Infection with Bonomia ostrae

Infection with *Perkinsus marinus*

Infection with *Microcytos mackini*

Infection with *Marteilia refringens*

Ostreid herpesvirus1 µvar (OsHV-1 µvar)

Taura syndrome

Yellowhead disease

White spot disease

Additional Designations

WOAH Reference Laboratory for Koi Herpes Virus (KHV) and Spring Viraemia of Carp Virus (SVCV).

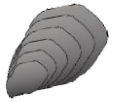
WOAH Collaborating Centre for Emerging Aquatic Animal Disease since 2019, with a remit to identify and manage new diseases that affect farmed and wild aquatic animals.

The UK's International Reference Centre for Antimicrobial Resistance (AMR) has been designated by the Food and Agriculture Organization of the United Nations (FAO) as the FAO Reference Centre for AMR. The Centre is led by three of the UK government's Department for Environment, Food and Rural Affairs' (Defra) Executive Agencies: Cefas, the Veterinary Medicines Directorate (VMD) and the Animal and Plant Health Agency (APHA).

Additional WOAH Listed Diseases [ISO 9001 accredited assays]

Infection with *Aphanomyces invadans* (epizootic ulcerative syndrome)

Infection with Tilapia Lake virus (TiLV)



Infection with abalone herpesvirus (AbHV)

Infections with Xenohaliotis californiensis

Infection with *Aphanomyces astasci* (crayfish plague)

Infection with infectious hypodermal and haematopoietic necrosis virus

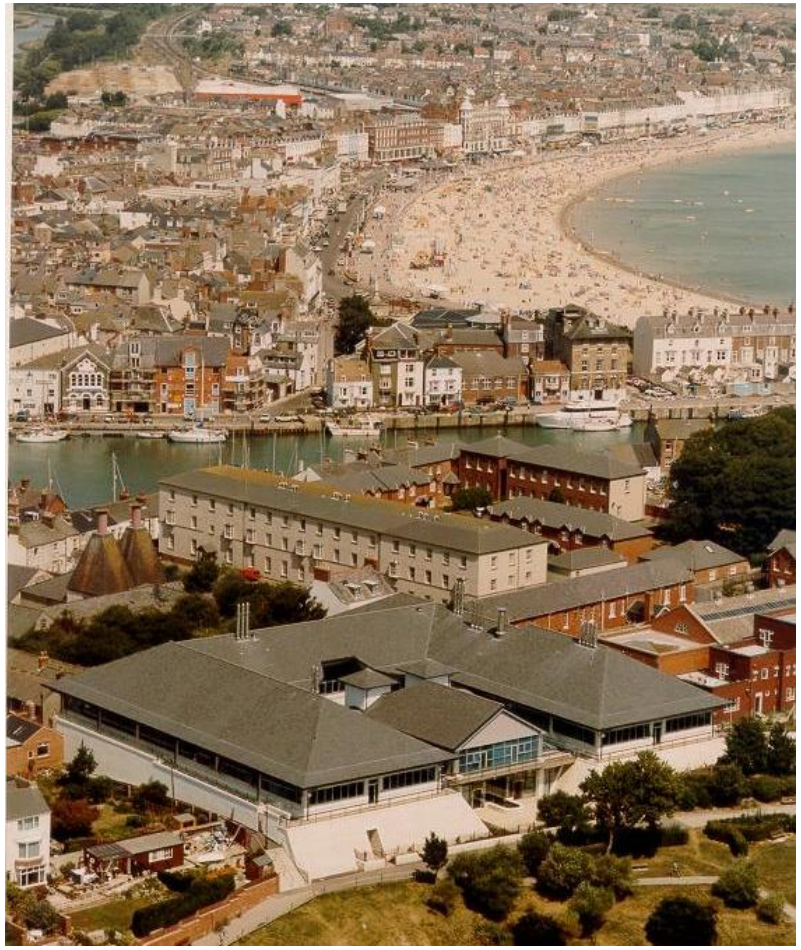
Infection with infectious myonecrosis virus

Infection with *Batrachochytrium dendrobatidis*

Infection with *Batrachochytrium salamandrivorans*

Infection with ranavirus

Cefas Weymouth Laboratory Facilities



The new Cefas Weymouth Laboratory was built in 1994 and replaced the old MAFF Fish Diseases Laboratory.

‘State of the Art’ facilities at the time.

The National Reference Laboratory (NRL) for Aquatic Animal Diseases

Multidisciplinary laboratory

- Diagnostic and Research Virology (1 and 5 staff respectively) with Tissue Culture facilities (1 staff)
- Histology and Transmission Electron Microscopy (5 staff)
- Bacteriology (2 staff)
- Diagnostic and Research Molecular Biology/Molecular facilitates (7 and 8 staff respectively - Developed since 1994
- Epidemiology

Fish

Molluscs

Crustacean

Clinical signs / Sampling

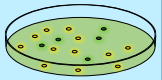
Microscopy

- Histopathology
- Electron microscopy

Virology

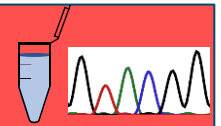
- Cell culture
- IFAT
-ELISA

Bacteriology



- Bacterial isolation
- Primary tests
- Secondary tests
- MALDI-TOF

Molecular biology



- PCR
- real time PCR
- Sanger sequencing
- *in situ* hybridization

CHAPTER 2.3.7.

RED SEA BREAM IRIDOVIRAL DISEASE

Table 5.1. Methods for targeted surveillance and diagnosis

Method	Targeted surveillance				Presumptive diagnosis	Confirmatory diagnosis
	Larvae	PLs	Juveniles	Adults		
Gross signs	d	d	d	d	b	d
Bioassay (virus isolation in cell culture) and identification by IFAT or PCR)	c	c	c	c	a	a
Direct LM	d	d	c	d	b	d
Histopathology	d	d	d	d	b	d
Transmission EM	d	d	d	d	b	d
Antibody-based assays (IFAT) of isolated virus or stamp-smear	c	c	c	c	a	a/b
PCR	c	c	c	c	a	a
Sequence	d	d	d	d	a	a

a = recommended; b = standard method; c = limited application;
d = not recommended

Surveillance

No recommended assays

Real-time Taqman qPCR (Mohr et al 2015)
RSIV RT F 5'-TGACCAGCGAGTTCCTTGACT-3'
RSIV RT R 5'-CAAGTCTGACCGTTGGTGATACC-3'
RSIV Probe
FAM-AACGCCTGCATGATGCCTGGC-TAMRA

Confirmation of disease

WOAH recommended
1-F 5'-CTCAAACACTCTGGCTCATC-3'
1-R 5'-GCACCAACACATCTCCTATC-3'

Megalocytivirus PCR (Rimmer et al 2012)
C1105 5'-GGTTCATCGACATCTCCGCG-3'
C1106 5'-AGGTCGCTGCGCATGCCAATC-3'
C1073 5'-ATGCCGTGACCTACTTTGCC
C1074 GATCTTAACACGCAGCCACA

Focus: Molecular Diagnostic Facilities

4 rooms with unidirectional workflow

- Sample preparation and nucleic acid extraction
- Reagent and PCR/RT-PCR setup
- PCR amplification
- Post PCR product analysis (electrophoresis and sequencing)

Sample preparation and nucleic acid extraction

Sample preparation and digestion.

- FastPrep tissue homogeniser or
- Clarified samples also come from virology.
- Large oven or smaller agitated heating blocks for tissue digestion
- Cabinets and work areas cleaned/UV treated before and after use.

Sample preparation and nucleic acid extraction (cont.)

Fully automated nucleic acid extractions

- 2 x EZ-1xl Biorobots with 14 tube capacity
- 2 x Qiacube HT Biorobots with 96 well capacity.

Reagent and PCR/RT-PCR setup

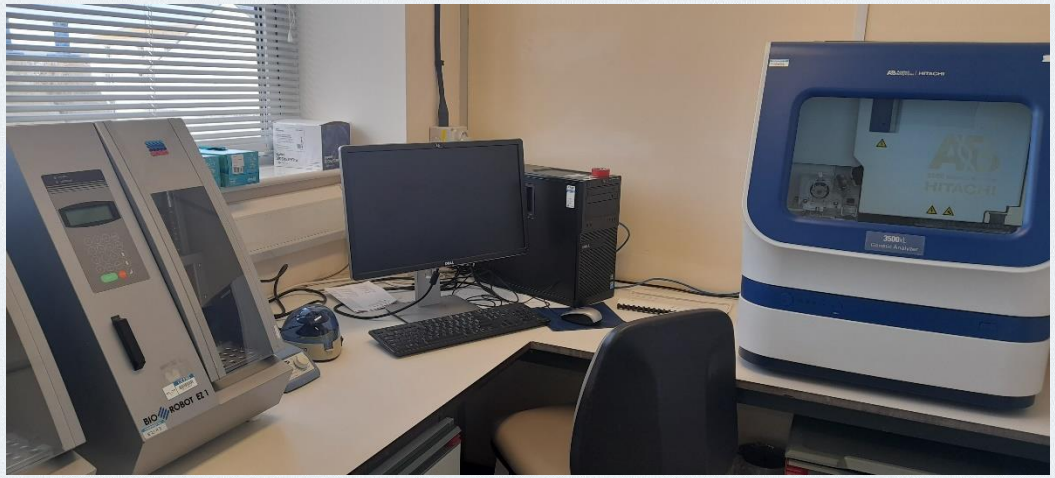
- PCR enclosure with UV disinfection
- Dedicated fridge and freezer for the PCR/RT-PCR reagents

No +ve control material

PCR amplification

- -80°C storage of +ve control materials
- PCR enclosure for the addition of the positive control
- Conventional and real-time PCR thermal cyclers

Post PCR product analysis (electrophoresis and sequencing)



- Standard electrophoresis and gel documentation equipment
- 3500xl Genetic analyser (24 capillaries)
- 2x EZ-1 BioRobts for extraction of positive control material
- Registered under GMO regulations for cloning

Services (and Challenges encountered in assisting Members States)

- Provision of positive control materials
  - Live or heat inactivated virus
  - Plasmid DNA with internal TAG sequence
  - RNA transcripts with internal TAG sequence
- Provision of fish cell lines
- Provision of advice on diagnostic methods with some limited training
- Confirmation for suspect cases of SVCV, KHV, CEV and others