MAFF PROJECT FC1136: RESEARCH ON THE IDENTIFICATION, DIAGNOSIS AND SIGNIFICANCE OF NOTIFIABLE AND EMERGING VIRUS DISEASES OF FISH AND SHELLFISH

by P. F. Dixon (contract leader), A. Betts, C. Davis, R. -M. Le Deuff, P. Martin, D. Stone, G. Taylor and K. Way

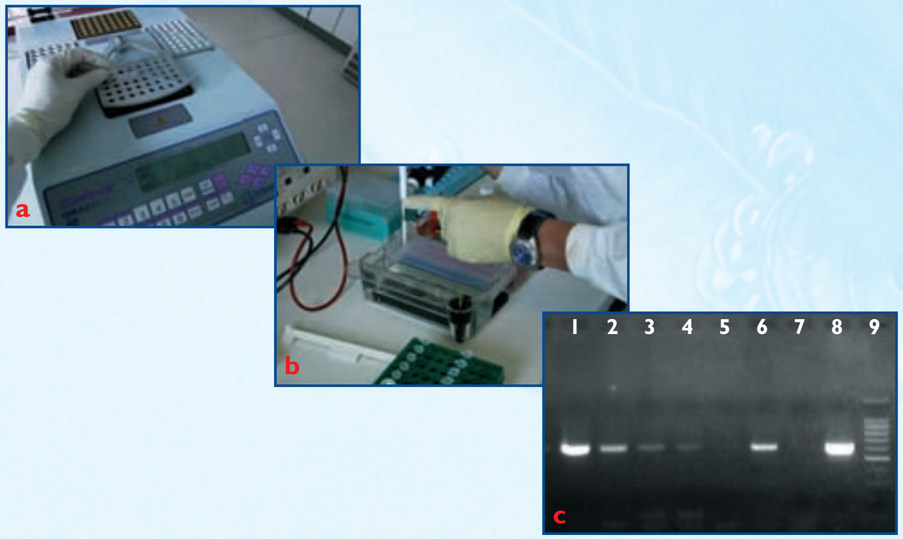
CEFAS
The Centre for Environment, Fisheries & Aquaculture Science

Development and validation of diagnostic techniques

- The aim of this area of research is to provide information to enable notifiable virus diseases to be rapidly and unequivocally identified, so that control measures can be put in place with the minimum of delay.
- Research is undertaken to provide rapid, sensitive and specific diagnostic methods that have been validated to the satisfaction of ourselves and the wider scientific community. We are then in a strong position when negotiating with the EU as to whether or not certain diagnostic methods should be used throughout the Community.

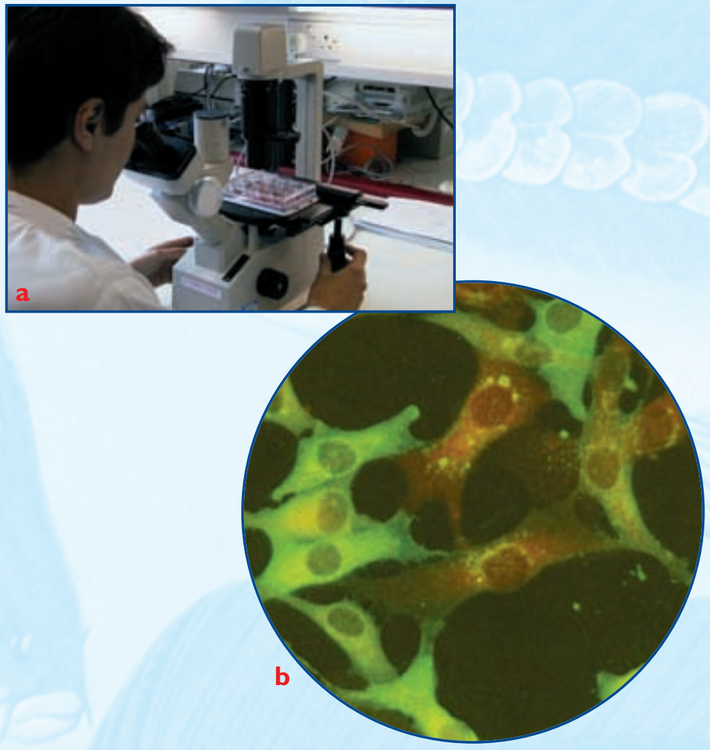
Diagnosis of notifiable virus diseases

- Important notifiable diseases of fish include viral haemorrhagic septicaemia (VHS) and infectious haematopoietic necrosis virus (IHN) which mainly affect salmonids and spring viraemia of carp (SVC) which mainly affects cyprinids.
- The standard method of detection of viruses is by isolation in cell culture followed traditionally by serological identification. However, techniques based on the detection of the virus genome are now being used more routinely.
- Techniques such as the polymerase chain reaction (PCR), complement the traditional methods and in addition to being rapid and sensitive, the PCR can provide information on the genetic relationships of viruses.



Detection of virus nucleic acid by PCR. Amplification of virus sequences using a robot thermocycler (a). Agarose gel electrophoresis of PCR products (b). Visualisation of amplified virus sequences as white bands on the gel (c).

- Other methods such as *in situ* hybridisation (ISH) are now under development in our laboratory which allows the direct detection of virus nucleic acid in fish tissue sections. The visualisation of virus components by ISH can be compared to the older techniques of immunohistochemistry or the fluorescent antibody test (FAT), but ISH appears to be more sensitive.
- As molecular biology techniques are more sensitive than traditional serological techniques, they are being used more in disease investigations or for disease monitoring in different laboratories e.g. for the detection of virus carrier fish.
- Conflicting results may be obtained by molecular and conventional techniques which can create difficulties for those who have to administer disease control policies.
- The PCR and ISH are not accepted by the EU or by our own Fish Health Inspectorate as primary diagnostic methods, although PCR is accepted as an epidemiological tool.
- Research is needed to validate the PCR and the ISH as diagnostic tools and to determine the limitations and benefits of both cell culture isolation and the new molecular based techniques.



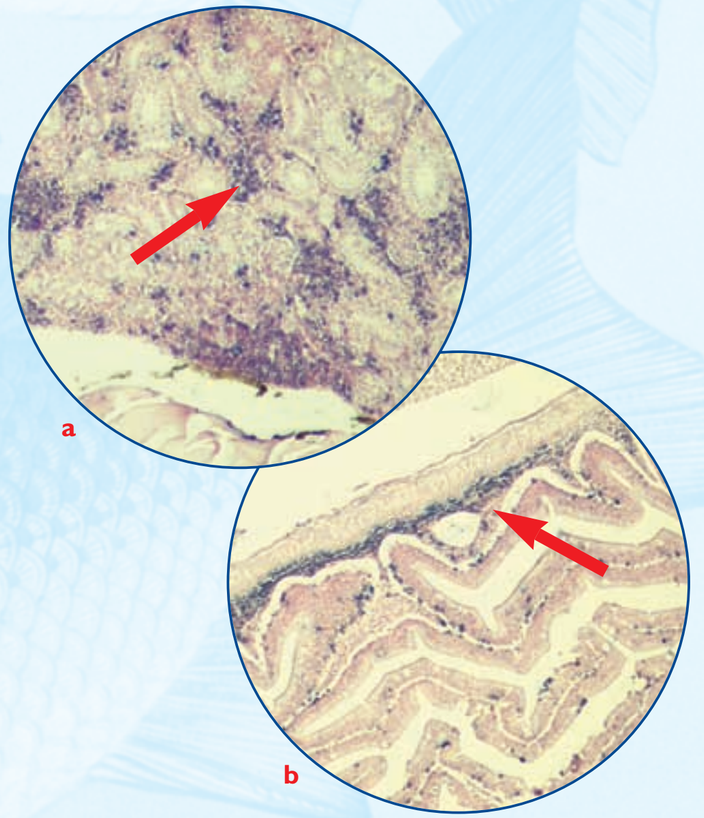
Isolation of viruses on tissue culture (a) is the standard reference diagnostic method. Traditionally it is accompanied by a confirmatory identification test, such as FAT (b) or ELISA.

Emerging viruses

- New viruses continue to emerge in cultured fish and shellfish worldwide. In recent years serious diseases have been caused by nodaviruses in marine fish and by iridoviruses in both marine and freshwater fish. A more recently described herpesvirus in koi carp, is a potentially serious pathogen.
- It is prudent to be in a position to be able to diagnose those diseases should they occur in the UK.
- To achieve this, conventional diagnostic methods as well as other molecular based methods (mainly based on PCR) developed in other laboratories are evaluated at the CEFAS Weymouth Laboratory.
- In addition to these potential threats to cultivated and wild fish, we are also involved in work on viruses associated with losses of shellfish in other countries.
- We are collaborating with other European laboratories on the validation of diagnostic methods for the oyster herpesvirus (OHV) by PCR and ISH.

Questions

- Are the existing techniques accurate and quick enough; do they need to be improved or do we need to adopt other techniques?
- Can we rely on the accuracy of the new techniques with respect to their sensitivity, specificity and reproducibility?
- Is it always relevant to use one of the new techniques and what would be the benefit when compared with reference methods?
- Do we need to develop new diagnostic methods for new and emerging diseases?
- Are we up to date with state of the art techniques?



In situ hybridisation on fish tissue sections. The localisation of virus DNA/RNA is visualised by the development of a purple-blue colour, corresponding to the binding of a labelled probe. a) Acute SVC infection in kidney section. b) Early SVC virus infection in the gut.