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KHVD, Diagnosis, Control, Research and Future in The Netherlands and Europe

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In recent years koi herpesvirus disease (KHVD) has quickly spread over Europe. In several European countries the first KHVD cases were observed in 2002 (Italy, Denmark) and 2003 (France, Austria, Switzerland). In contrast to Western Europe, where koi and carp are mainly kept as ornamental fish, in Eastern Europe carp is farmed for consumption and KHVD could have a huge impact on the carp industry. At the moment however, Eastern Europe still seems free from KHVD. However, common carp exported from Poland to Germany were shown to be KHV positive. The highest incidence of KHVD cases in Europe are found in Germany, England and the Netherlands.

Germany has had over 250 cases since 2002. England had 36 cases in 2002, and more cases followed in 2003.

In the Netherlands a steady increase was seen from 2 KHV positive cases in 2001 up till 27 cases in 2003 (Table 1).

Concern for these countries is that KHVD could possibly not only be restricted to koi-trading and private ponds but already be spread into the natural environment.

At the moment however there is still no evidence for this. As KHVD is not notifiable for the European Union (EU), the control on spread of the disease within the EU is minimal.

The majority of the KHV cases in the Netherlands were detected by PCR (Gilad *et al.*, 2002), with or without using gill histopathology as a confirmatory method (Fig. 2). In a few cases the herpes virus could be cultured successfully on Epithelioma Papilloma Carposum (EPC) or Koi Fin-1 (KF-1) cells (Fig. 1).

In 2001 we succeeded in isolating the virus for the first time in the Netherlands from a clinical diseased koi. The inoculated sample of gills and pooled internal organs showed cytopathic effect (CPE) at the 2nd passage on EPC cells

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Table 1. KHV in The Netherlands.

Year	KHV cases	Host
2001	2 positive cases out of 6 (PCR): 1 virus isolation	Only koi affected. Up till now no cases in carp
2002	6 positive cases out of 30 (PCR): 1 virus isolation	
2003	27 positive cases out of 63 (PCR/hist.)	

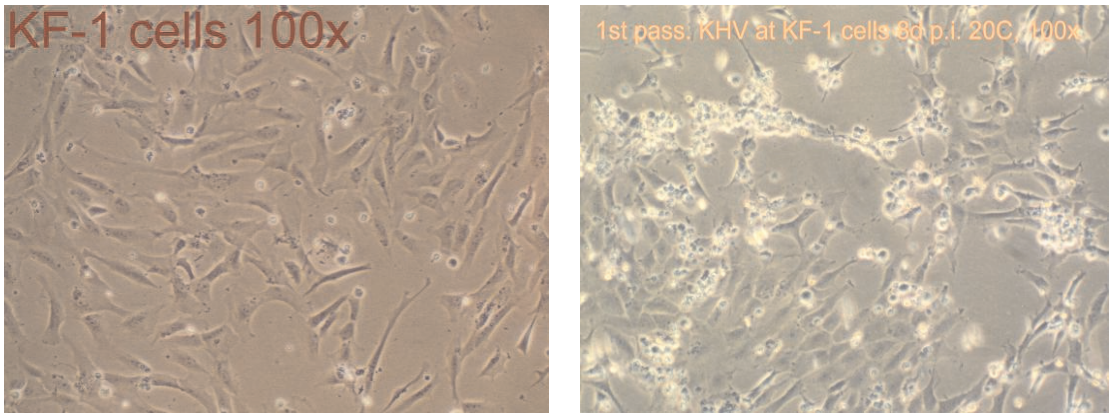

Fig. 1. Virus isolation of KHV on KF-1 cells. Left, control KF-1 cells. Right, 8 days post infection at 20℃. Confirmation of KHV with EM and PCR.

Unfortunately the sensitivity of viral isolation is inadequate for diagnostics of KHV. Additional methods, as PCR, are required for correct diagnosis of the disease.

Currently effort is put into developing alternative detection methods as immuno-fluorescence tests (IFT) to support diagnostics of KHVD (Fig. 2).

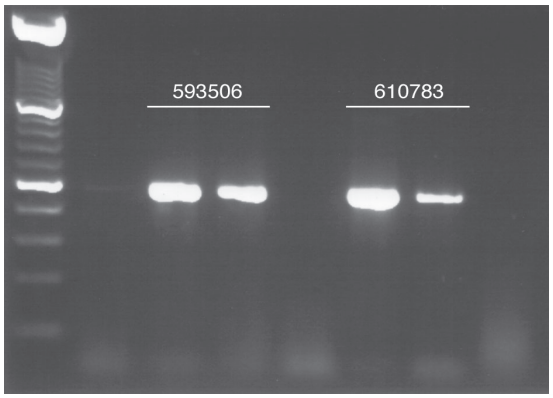

Fig. 2. Example of detection of KHV with PCR method according to Gilad *et al*. 2002. Two samples, for each sample gill tissue (left) and pooled internal organs (right). DNA isolated from gills and internal organs with commercial QIAgen kit.

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