Serology for KHV surveillance

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20th Annual Finfish diseases NRL workshop

Centre for Environment Fisheries & Aquaculture Science

Cefas

Why serology for KHVD (CyHV-3) ?

- PCR most appropriate for clinical diagnostics
- But less so for surveillance (sub clinical) -
- Persistence/latency
- Low levels of virus
- Irregular distribution
- Poor growth in cell culture

Development of a KHV Elisa

Reactivation of koi herpesvirus infections in common carp *Cyprinus carpio*

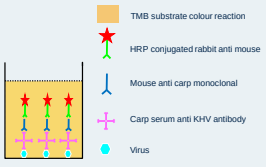
S. St-Hilaire[1,3,*], N. Beevers[1], K. Way[1], R. M. Le Deuff[1], P. Martin[2], C. Joiner[2]

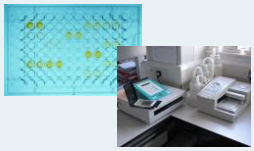
An Enzyme Linked Immunosorbent Assay (ELISA) for Detection of Antibodies to the Koi Herpesvirus (KHV) in the Serum of Koi *Cyprinus carpio*

Mark A. Adkison[1], Oren Gilad[2] and Ronald P. Hedrick[1*]

An Enzyme-Linked Immunosorbent Assay (ELISA) for Detection of Antibodies to Channel Catfish Virus (CCV) in Channel Catfish

KHV Elisa

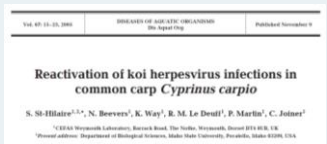




- Used pooled positive and negative controls
- Sample considered positive if:

OD reading of 1/1600 dilution > av. neg control + 3 SD

Development of KHV Elisa



Reactivation of koi herpesvirus infections in common carp *Cyprinus carpio*

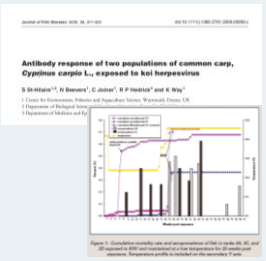
S. St-Hilaire[1,3,*], N. Beevers[1], K. Way[1], R. M. Le Deuff[1], P. Martin[2], C. Joiner[2]

- Study on persistence, reactivation and transmission
- 10-25% surviving fish had antibodies to KHV

Antibody response

- Assessment of seroprevalence over time
- Seropositivity in 20-60% experimentally infected fish
- Still detectable at 7 months
- Observed at non permissive temperatures

- Tested and observed cross reactivity with CyHV-1 (carp pox) positive experimental sera samples from USA group at low dilutions (1/200, 1/400)



Antibody response of two populations of common carp, *Cyprinus carpio* L., exposed to koi herpesvirus

S St-Hilaire[1,2], N Beevers[1], C Joiner[1], R P Hedrick[3] and K Way[1]

Further test optimization, validation and a serological survey


Koi herpesvirus: distribution and prospects for control in England and Wales

N G H Taylor, P F Dixon, K R Jeffery, E J Peeler, K L Denham and K Way

- Optimisation of coating antigen, primary and secondary (conjugated) detecting antibodies.
- Pooled +ve and –ve controls in duplicate per plate.
- Assay validity thresholds determined –

  Mean absorbance of +ve > 0.466 and < 0.731: Mean absorbance of –ve > 0.006 and < 0.103.
- Tests on serum samples from carp experimentally infected with CyHV-1 (cross reaction threshold 1/400).
- Assay and operator repeatability tests performed within lab and externally.
- 809 samples tested from sites with known history of presence or absence of KHV and or carp pox.

Further test optimization, validation and a serological survey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- Change from use of a specific OD value to sample to positive (S/P) ratio for better diagnostic performance
- High +ve/-ve cut-off - S/P 13.67% - D-Sp 98%; D-Sn 72%
- Low +ve/-ve cut-off - S/P 6.02% - D-Sp 90%; D-Sn 80%
- With D-Sn in the range of 72-80% the assay was acceptable at the population level.

- With the listing of KHV under 2006/88/EC and implementation in UK law in 2007, the elisa was used in a survey to establish the geographic distribution and prevalence of KHV in English and Welsh farms and fisheries, undertaken in 2007, to asses options for categorisation and control.

Serological survey


Koi herpesvirus: distribution and prospects for control in England and Wales

N G H Taylor, P F Dixon, K R Jeffery, E J Peeler, K L Denham and K Way

- 30 fish samples were taken from:
- 82 farm sites, 71 "high risk" fisheries and 12 consignments of imported koi from SE Asia countries

	Farms sampled	Positive farms	Fisheries sampled	Positive fisheries
South West	18	2 (11%)	8	3 (40%)
Southern	13	1 (8%)	8	0
Thames	8	0	10	3 (30%)
Anglian	17	0	14	4 (30%)
Midlands	16	0	10	6 (60%)
Wales	1	0	5	2 (40%)
North West	2	0	11	7 (64%)
North East	7	0	5	1 (20%)
Total	82	3 (4%)	71	26 (37%)

Table 2 Imported consignments of koi carp tested for koi herpesvirus antibody by EIA

Country of origin	Consignments tested	Antibody positive consignments
Malaysia	9	1
Thailand	9	1
China	2	1
Sri Lanka	1	1
Indonesia	1	1
Hong Kong	1	1
Singapore	11	0
total	12	6

CTL - Commercially offered KHV antibody elisa test

10% of population or 30 fish samples recommended

Year	No. populations +ve / no. tested	Total No fish tested
2007-2008	19/29	505
2008-2009	16/22	1604
2009-2010	12/27	914
2010-2011	9/16	1420
2011-2012	8/14	212
2012-2013	0/2	121

Issues of KHV Elisa

- Known cross reaction with CyHV-1 (carp pox) at 1/200 and 1/400 dilutions.
- Hence 1/1600 working dilution.
- Increased dilution leads to reduced sensitivity
- Likely cross reaction with other herpesviruses such as:

  KHV variants (95-98% id).

  Other herpesviruses (<95% identity) of which 5 identified between 2000 and 2014.

  AngHV-1

Potential improvements - ongoing

- Use of alternative antigens specific to CyHV-3 to reduce CyHV-1 cross reaction:

Wasa et al., University of Stirling.

Synthetic peptide synORF84 (capsid protein)

Recombinant proteins to rORF62 (OTU-like cysteine protease domain) and rORF68 (myosin related)

- % positive responders to rORF62 and 68 (95% and 90%) was higher than synORF84 (76%) but synORF84 more specific in cross reactivity with CyHV-1.

Kattlun et al., University of Veterinary Medicine, Vienna

pORF81 DNA vaccine (potential major envelope protein)

Transcription confirmed but no antibody response detected in sera of vaccinated fish - not antigenic or below detection level.

Potential improvements - ongoing

- synORF84 more specific for CyHV-3 but not as sensitive - background issues with coating.

  Potential for use of cocktails of specific antigens.

  Attempts ongoing to reduce background noise

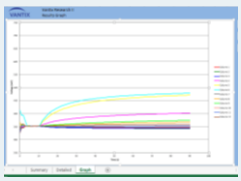
Potential improvements

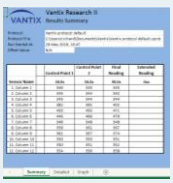
Investigating alternative methodologies - Vantix system

- Electrochemical sensor measures potential rather than colour change
- Reported advantages of speed, sensitivity, complex samples (blood).

Potential improvements

15-20 minutes vs 3.5 hour





Summary

- KHV antibody elisa developed and optimised and subjected to relatively thorough validation.
- Suitable for population level assessment
- Used to good effect in control policies in E&W
- More we find out about additional herpesviruses more improvement and re-validation required.
- Obtaining or generating reference antisera not always easy (non culturables)