VALIDATION OF A SERONEUTRALISATION TEST ALLOWING THE DETECTION OF ANTIBODIES SPECIFIC TO KOI HERPESVIRUS (KHV)

Joëlle CABON[1], Jeannette CASTRIC[1], François LAMOUR[1], Marek MATRAS[2] and Thierry MORIN[1]

[1] Ploufragan-Plouzané Laboratory, Unit Viral Disease of Fish, Brest, France

[2] Department of Fish Diseases, National Veterinary Research Institute, Pulawy, Poland

Koï Herpesvirus (KHV or $CyHV_3$)

➥ Etiological agent of a **contagious disease regulated in Europe** (Directive 2006/88/EC)

➥ **Affecting common carp and varieties** such as koï and ghost carp

➥ Apparition in Europe, the US, Israel and Japan in the late 90s. Geographical extension highly favored by the international trade

➥ **Symptomatic infections between 18 and 28 °C**: marked clinical signs are large skin ulcers, excess mucus production and hemorrhages in the fins.
Mortality rates reaching 100%

➥ Outside this temperature range, **persistance in a latent state** in infected hosts which remain asymptomatics, contributing to the virus spreading.

➥**KHV detection in these healthy carriers is difficult using the direct diagnostic methods recommended by the OIE**

Objective

Validate an indirect and non-lethal seroneutralisation (SN) test allowing the detection of KHV specific antibodies from sera of carps

Principle:

❶ Blood sampling

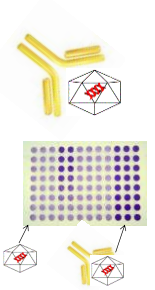
❶' Virus production and titration on CCB.
Isolate 07/108b – passage >70

❷ Neutralisation reaction (day 0) : decomplemented sera + virus during 18h at 5 °C

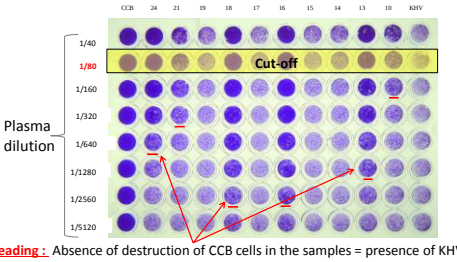
❸ Transfer on CCB cell (day 1)
Incubation 7 to 10 days at 24 °C

❹ Staining (day 7 to 10)
with cristal violet

❺ Validation of the controls and interpretation



Interpretation



Reading : Absence of destruction of CCB cells in the samples = presence of KHV neutralizing antibodies.
The titre of a serum is the inverse of the dilution giving 50% protection of the cell monolayer compared to the control virus and negative serum.

Controls to include:
① sera positive for KHV, ② sera positive for CyHV1, ③ sera negative for KHV and CyHV1, ④ CCB cells alone, ⑤ KHV + CCB cells

Samples used

➥ **204** KHV negative or positive sera:

Sample identification	Total number	Origin	Expected serological status
NEG1 to NEG60	60	3 French **farms with no history of KHV**	KHV negative
$CyHV1_1$ to $CYHV1_5$	5	**Experimental contamination** with CyHV1 isolate HH15	KHV negative CYHV1 positive
ITA1 to ITA5	5	Samples provided by Dr G. Bovo (Italia) from **naturally contaminated carps**	KHV negative CyHV1 positive
CCV1 to CCV5	5	**Sera positive** for Chanel Catfish Virus specific antibodies	KHV negative CCV positive
HVA1 to HVA5	5	Sera positive for Herpesvirus Anguillae specific antibodies	KHV negative HA positive
ENG1 to ENG5	5	**Samples from carps tested positive in virology** for a KHV English isolate provided by Dr S. Bergmann (Germany)	KHV positive
TAIW1 to TAIW5	5	Samples from carps tested positive in virology for a KHV Taiwanese isolate provided by Dr S. Bergmann (Germany)	KHV positive
US1 to US10	10	Experimental contamination with KHV US isolate F98/50	KHV positive
EXP11 to EXP30	20	Experimental contamination with KHV French isolate 07/108B	KHV positive
POL21 to POL85	84	Sera from farms with clinical signs of KHV provided by Dr M. Matras (Poland)	KHV negative or positive ?

Analytical and diagnostic performance

Samples identification	Number of samples	Expected KHV Status	% of KHV positive samples obtained using the SN test (%)
NEG1 to NEG60	60	-	1.7
$CyHV1_1$ to $CYHV1_5$	5	-	0
ITA1 to ITA5	5	-	0
CCV1 to CCV5	5	-	0
HVA1 to HVA5	5	-	0
ENG1 to ENG 5	5	+	80
TAIW1 to TAIW5	5	+	100
US1 to US10	10	+	100
EXP11 to EXP30	20	+	100

➢Positive detections obtained with sera of carps infected by various strains of KHV virus : European (French 07/108B, English isolate), Taiwanese, American (F98/50).

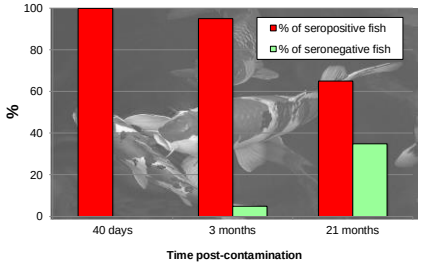
➢Absence of KHV neutralization with sera against CyHV1, Chanel Catfish Virus and Herpesvirus Anguillae.

Diagnostic specificity	Diagnostic sensitivity	Relative accuracy	Repeatability	Intra-laboratory reproducibility
98.75% (n=80 sera)	**97.5%** (n=40 sera)	**98.4%**	**100%** (n=10 sera, 5 times, identical analytical sequences)	**100%** (n=10 sera, 9 different operators and/or time)

anses

Application to KHV experimentally infected Koï carps

Inoculation by bath, 24°C
KHV strain 07/108B
20 sera analyzed /each sampling point



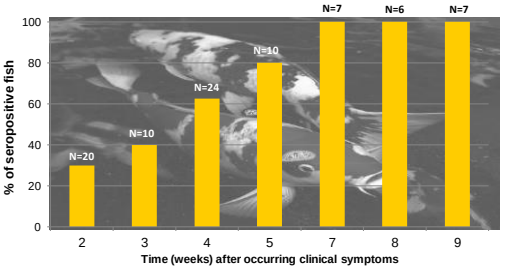
➥ 100% of detection of KHV neutralizing antibodies 40 days post-infection.

At 21 months post-infection, 65% of the population remained positive.

anses

Application to KHV naturally infected Koï carps

15 Polish carp farms (Dr M. Matras)



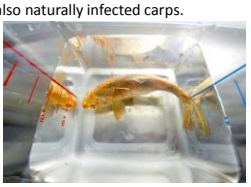
➥ More than 50% of the individuals were positives 4 weeks after the appearance of the first symptoms and 100% after 7 weeks *(Matras et al. Bull Vet Inst Pulawy 56, 127-132, 2012).*

anses

Conclusions

➥ This technique of neutralisation offers guarantees of reliability and robustness that allow its application in confidence.

➥ It has proven its efficiency to detect KHV specific neutralizing antibodies in experimentally but also naturally infected carps.



➥ At a farm scale and applied to a sufficient number of sample, it can be used as indirect diagnostic technique to determine the health status of fish for KHV, without sacrificing valuable animals for the farmer (parents).

anses

Thank you for your attention

We sincerely thank **Dr. S. BERGMANN** (FLI, Germany), **Dr G. BOVO** (IZSV, Italy) and **Dr. M.MATRAS** (National Institute of Vet Res Pulawy, Poland) for providing samples.

The PVP team

anses