Susceptibility of CCB cell line to different fish viruses

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In spite of the establishment of modern techniques like PCR for virus detection, virus isolations in susceptible fish cell lines are still the basis for international certification of most notifiable viral fish diseases mentioned by the OIE and the European Union (OIE, 2000; European Commission, 1996). Cell cultures may change in susceptibility (Lorenzen et al., 1999, unpublished own findings,). If no regular susceptibility checks are done in the laboratory, using an alternative sensitive cell culture might therefore increase the chance of isolating a certain virus. The CCB (common carp brain) cell line, developed by and cultured according to Neukirch et al., 1999, was originally developed for the diagnosis of koi herpesvirus (KHV). Moreover, we isolated other viruses from diseased koi in CCB cells. Besides KHV, myxoviruses, birna- and/or reoviruses (not all isolates were identified) and rhabdoviruses (spring viraemia of carp virus, SVCV) were obtained (Neukirch & Kunz, 2001). The susceptibility of CCB cells was also investigated for viruses isolated from golden ide, European eel and salmonids. CCB cells and the standard cell lines used in our laboratory: Epithelioma papulosum cyprinid, EPC (Tomasec and Fijan, 1971), Chinook Salmon Embryo, CHSE (Fryer et al., 1965) and Eel kidney, EK-1 (Chen et al., 1982) for the different viruses were inoculated with the following viruses: infectious pancreatic necrosis virus (IPNV, strains Sp, Ab and Ni), viral haemorrhagic septicaemia virus (VHSV, serotype I, II and III), infectious haematopoietic necrosis virus (IHNV), golden ide reovirus (GiRV), chum salmon reovirus (CSV), herpesvirus anguillae (HVA), and another eel virus designated A_1B virus. Non-infected cell lines were incubated as negative controls. Two passages were carried out in the cell lines before quantification of virus infectivity by titration of supernatants in 10-fold dilution steps (Mayr et al., 1977). Table 1 summarizes the inoculated viruses used, and results concerning titration of virus infectivity obtained in both the routine cell lines and the CCB cell line.

The results demonstrate the high susceptibility of the CCB cell line to a wide range of different fish viruses. All rhabdoviruses included in the investigations could be multiplied in this cell line with infectivity titers similar to those obtained in the routinely used cell lines. Chum salmon reovirus and the IPN viruses grew equally well in CCB cells compared to the standard cell lines. The lower infectivity titers in CCB cells may be attributed to an incomplete adaptation of the vi-

Viruses	Type/strain	Infectivity titers in Standard used cell lines		CCB* cells
		Cell line	Titer	Titer
Rhabdoviridae				
VHSV	Type 1(07.71)	EPC[1]	7.75[2]	6.50
	Type 2	EPC	4.75	3.75
	Type 3 (23.75)	EPC	6.00	6.00
IHNV	(32.87)	EPC	4.50	4.25
Birnaviridae				
IPNV	Sp	EPC	6.50	7.50
	Ab	CHSE[3]	5.75	5.25
	Ni	CHSE	6.70	5.75
Reoviridae				
CSV	(017.94)	CHSE	4.75	3.25
GiRV		EPC	n.d.	Weak CPE
Herpesviridae				
HVA		EK-1[4]	7.2	Neg.
Orthomyxoviridae				
A_1BV		EPC	n.d.	Weak CPE

* Established for the diagnosis of Koi Herpes Virus; 1) Epithelioma papulosum cyprini (Tomasec and Fijan, 1971); 2) Log_{10} $TCID_{50}$/0.2 ml; 3) Chinook Salmon Embryo (Fryer et al., 1965); 4) Eel Kidney (Chen et al., 1982).

Table 1. Susceptibilities of CCB cells and standard used cell lines to various fish viruses.

ruses to the cell line rather than to a lower susceptibility of the CCB cell line. After two passages of GiRV and the eel virus A_1B only weak and retarded cytopathic effects (CPE) in CCB cells with negligible infectivity could be observed. Herpesvirus anguillae did not replicate in CCB cells. Therefore, we suggest, that in addition to the viruses isolated from carp, the CCB cell line is suitable for the propagation of at least salmonid rhabdoviruses and some IPN viruses, too.

Rhabdoviruses were kindly obtained from P. de Kinkelin, INRA, Jouy-en-Josas, France. IPNV strains, type Ab and Sp were kindly obtained from W.Ahne, University of Munich, Germany. The IPNV strain Ni was kindly obtained from K.E. Christie, NORBIO A/S, Bergen, Norway. The CSV strain was kindly obtained from S. LaPatra, Research Dept. Clear Springs Foods, Buhl, USA. HVA originated from CIDC-Lelystad (Davidse et al.,

1999; Haenen, author), and GiRV and A_1BV from the School of Veterinary Medicine (Neukirch, author).

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