

Presence of eel viruses in eel species from various geographic regions

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EVEX (Eel-Virus-European-X), HVA (*Herpesvirus anguillae*), and EVE (Eel Virus European) were detected in wild and farmed European eels (*Anguilla anguilla* L.) from the Netherlands, EVEX and EVE from farmed eels from Italy, and EVEX from wild eels from Morocco. EVEX was also isolated from wild New Zealand eel (*A. dieffenbachi*). Elvers (*A. anguilla*) collected from eel farms in the Netherlands were mainly infected with HVA. An unknown picornavirus was isolated from healthy wild European eel from The Netherlands, and New Zealand eel (*A. dieffenbachi*) from New Zealand. European eels from Northern Ireland showed clinical signs of virus infections although no viruses were detected in these samples.

Fish viruses can cause disease or even mortality when fish are under stressful conditions. Eels can suffer various viral infections. Firstly, infections with EVA (Eel-Virus-America) and EVEX have been described. Both viruses are serologically related (Kobayashi & Miyazaki, 1996). EVA was first discovered in Japan in 1974, in a shipment of American elvers, which had been stocked in Cuba (Wolf, 1988). Another virus, which was isolated in a shipment from France to Tokyo, was named EVEX because of its European origin (Sano et al., 1977).

Another eel virus is EVE (Eel Virus European). Egusa (1970) described a new disease of Japanese eels (*Anguilla japonica*), called branchionephritis, with mortalities up to 50%. The eels had had contact with newly imported infected European eels (*A. anguilla*). In 1976 the EVE (European Virus of Eel) was isolated for the

first time (Sano, 1976) from European eels, showing severe renal pathology. The virus appeared to be serologically close by related to the French isolate of Infectious Pancreatic Necrosis Virus (IPNV), d'Honnincthun (Sano et al., 1981). Okamoto et al. (1983) regrouped EVE to group III of IPNV, together with the Danish isolate of IPNV, strain Ab (Jørgensen, 1971). The French isolate was placed in group II of IPNV, by the same authors. Hedrick et al. (1983) stated, that both EVE and IPNV type Ab were close related, but unique virus strains. EVE was pathogenic for Japanese eels, but not for rainbow trout fry (Sano et al., 1981).

Another eel virus is HVA *Herpesvirus anguillae*. In Asia, (HVA) it was isolated in 1985 from diseased Japanese eel *Anguilla japonica* and European eel *A. anguilla* (Sano et al. 1990), showing skin and gill erythema and necrosis of skin, gills, and liver. HVA is known to occur

also in farmed European eel *A. anguilla*, where it has caused considerable disease problems (Davidse et al., 1999; Haenen et al., 2002).

The aim of this study was to investigate the presence of viruses in eel populations from various geographic regions.

Live eels (25 lots) originating from 9 different countries (Table 1) were collected via an eel importer at Schiphol airport (The Netherlands). On the day of arrival they were anaesthetized with 300 ppm MS222 (3-aminobenzoic-acid-ethyl-ester methane sulphonate salt, Sigma, St. Louis, USA). Spleen, gills, kidney and liver were sampled and stored on dry ice. For virus isolation, at the Fish Diseases Laboratory (CIDC-Lelystad), samples of organs were homogenized with sterile medium and sterile sand, and inoculated on three cell-lines: RTG-2 - Rainbow Trout Gonad cells, FHM - Fat Head Minnow cells, and EK-1 - Eel Kidney cells, at 15°C, 20°C, and 26°C respectively, according to standard procedures (Davidse et al., 1999). In case of cytopathic effect, the infected cell line was inspected by electron microscopy followed by immunofluorescence or immunoperoxidase methods in order to identify the virus (Wolf, 1988, Davidse et al., 1999).

Several viruses were isolated from the investigated samples. Predominantly, EVEX was detected in wild and farmed European eel from various geographic regions: The Netherlands, Italy, and Morocco. However, only the eels from Italy showed clinical signs, like haemorrhages and red skin areas. Apart from European eel, EVEX was also isolated from wild New Zealand eel from New

Zealand, although they had no clinical signs of disease.

HVA was detected in farmed glass eels (elvers of *A. anguilla*) in The Netherlands, and in farmed adult eels in Italy from pond culture. In all these cases, the eels showed clinical signs of disease, like reddening with petechial haemorrhages. HVA is known to be present in farmed Japanese eels (*A. japonica*) in Taiwan (Ueno et al., 1992, 1996; Chang et al., 2002) and in farmed European eels in The Netherlands (Davidse et al., 1999, Haenen et al., 2002). In this study, we isolated HVA for the first time from wild (adult) European eels, in The Netherlands. It supports the hypothesis of van Nieuwstadt et al. (2001) and Haenen et al. (2002), that HVA is wide spread.

The impact of the isolated picorna-like virus from both European eels from The Netherlands and New Zealand eel (*A. dieffenbachi*) from New Zealand is not known, as they did not show clinical signs of disease. The adult European eels from Northern Ireland showed clinical signs of disease, like petechial haemorrhages, and red skin areas, but no virus was isolated from these eels.

We isolated several viruses from three eel species from various regions. Even elvers, for restocking were found positive for virus (HVA). Jørgensen et al. (1994) studied elvers and eels (*A. anguilla*) from several European countries (Denmark, England, France and Sweden) for presence of virus. They isolated EVEX, EVA, IPNV (Infectious Pancreatic Necrosis virus), and herpes-like viruses. There is much trade in live eels on a global scale. Trade of eels from infected areas like The Netherlands, which is one of the leading eel trading countries (Heinsbroek & Kamstra,

Country / Location	Species	Clinical signs	Virus	Positive at incubation temp (°C)	Infection rate (no. of eels)
NETHERLANDS					
Lake Grevelingen	<i>A.anguilla</i>	-	EVEX	15 and 20	6 from 6
Lake Grevelingen	<i>A.anguilla</i>	+	HVA	20 and 26	1 from 10
Lake Grevelingen	<i>A.anguilla</i>	-	EVEX	15 and 20	1 from 2
Lake Grevelingen	<i>A.anguilla</i>	-	picorna-like	20	1 from 2
Lake Brasemer	<i>A.anguilla</i>	-	negative	-	0 from 10
Lake Brasemer	<i>A.anguilla</i>	-	negative	-	0 from 10
Lake Lauwers	<i>A.anguilla</i>	-	negative	-	0 from 10
Lake Lauwers	<i>A.anguilla</i>	+	HVA	20 and 26	10 from 10
Eel farm-1	<i>A.anguilla</i>	+	HVA	20 and 26	pooled elvers
Eel farm-2	<i>A.anguilla</i>	+	HVA	20 and 26	pooled elvers
Eel farm-3	<i>A.anguilla</i>	+	HVA	20 and 26	pooled elvers
ITALY					
Eel farm-4	<i>A.anguilla</i>	-	EVEX & EVE	15, 20 and 26	4 from 4
Eel farm-5	<i>A.anguilla</i>	+	EVEX	20 and 26	3 from 4
FRANCE					
Loire	<i>A.anguilla</i>	-	negative	-	0 from 4
Perpignan	<i>A.anguilla</i>	+	negative	-	0 from 3
MOROCCO					
Sebou	<i>A.anguilla</i>	-	EVEX	26	2 from 4
USA					
Virginia	<i>A.rostrata</i>	-	negative	-	0 from 4
CANADA					
St.Lawrence	<i>A.rostrata</i>	-	negative	-	0 from 4
NEW ZEALAND					
Tekawata	<i>A.dieffenbachi</i>	-	picorna-like	20	1 from 4
Tekawata	<i>A.dieffenbachi</i>	-	EVEX	26	1 from 4
IRELAND					
River Roosky	<i>A.anguilla</i>	+	negative	-	0 from 4
Lake Corrib	<i>A.anguilla</i>	+	negative	-	0 from 4
Lake Ennel	<i>A.anguilla</i>	+	negative	-	0 from 4
mixed origin	<i>A.anguilla</i>	+	negative	-	0 from 4
UNITED KINGDOM					
Lake Erne (North. Ireland)	<i>A.anguilla</i>	+	negative	-	0 from 4

Table 1. Eel viruses isolated from eels of different locations.EVEX: Eel-Virus European X, HVA: *Herpesvirus anguillae*, EVE: European Virus of Eel.

1995), could impose a risk to other eel populations by transmitting several eel viruses.

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