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REVIEW

Viral diseases of wild and farmed European eel *Anguilla anguilla* with particular reference to the Netherlands

Steven J. van Beurden[1,2], Marc Y. Engelsma[1], Ineke Roozenburg[1], Michal A. Voorbergen-Laarman[1], Peter W. van Tulden[1], Sonja Kerkhoff[1], Anton P. van Nieuwstadt[1], Aart Davidse[1], Olga L. M. Haenen[1,*]

[1]Central Veterinary Institute, Wageningen UR, PO Box 65, 8200 AB Lelystad, The Netherlands
[2]Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, PO Box 80.165, 3508 TD Utrecht, The Netherlands

ABSTRACT: Diseases are an important cause of losses and decreased production rates in freshwater eel farming, and have been suggested to play a contributory role in the worldwide decline in wild freshwater eel stocks. Three commonly detected pathogenic viruses of European eel *Anguilla anguilla* are the aquabirnavirus eel virus European (EVE), the rhabdovirus eel virus European X (EVEX), and the alloherpesvirus anguillid herpesvirus 1 (AngHV1). In general, all 3 viruses cause a nonspecific haemorrhagic disease with increased mortality rates. This review provides an overview of the current knowledge on the aetiology, prevalence, clinical signs and gross pathology of these 3 viruses. Reported experimental infections showed the temperature dependency and potential pathogenicity of these viruses for eels and other fish species. In addition to the published literature, an overview of the isolation of pathogenic viruses from wild and farmed *A. anguilla* in the Netherlands during the past 2 decades is given. A total of 249 wild *A. anguilla*, 39 batches of glass eels intended for farming purposes, and 239 batches of farmed European eels were necropsied and examined virologically. AngHV1 was isolated from wild *A. anguilla* yellow and silver eels from the Netherlands from 1998 until the present, while EVEX was only found sporadically, and EVE was never isolated. In farmed *A. anguilla* AngHV1 was also the most commonly isolated virus, followed by EVE and EVEX.

KEY WORDS: Anguillid herpesvirus 1 · Eel virus European · Eel virus European X

INTRODUCTION

Freshwater eels of the genus *Anguilla* have extraordinary catadromous lifecycles, with the spawning grounds of some species located in the ocean several thousands of kilometres away from their freshwater growth habitats in lakes and rivers of the mainland (van Ginneken & Maes 2005). The wild freshwater eel stocks have shown strong declines worldwide since the 1980s (Dekker et al. 2003, Stone 2003). The cause of the decline is unknown, but is probably multifactorial, with suggested factors including pollution, habitat loss, fisheries, migration barriers, and diseases (Dekker 2004). Indeed, the swimbladder nematode *Anguillicoloides crassus* (Székely et al. 2009), several pathogenic bacteria (Esteve & Alcaide 2009) and certain viruses (Haenen et al. 2009) have been suggested to play a contributory role in the decline of the wild European eel *Anguilla anguilla* stock.

*Corresponding author. Email: olga.haenen@wur.nl

Anguilla anguilla and the Japanese eel *A. japonica* are traditionally consumed in several European countries and Japan, respectively (Heinsbroek 1991). Historically, eels for consumption were wild-caught, but European eel fisheries have recently been subject to limitations because of the population decline (Council of the European Union 2007). In Japan, eel farming for consumption purposes started at the end of the 19th century. Eel farming has developed from non-intensive polyculture in outdoor ponds to intensive indoor culture in greenhouses since the 1970s. Eel farming in Europe has its origin in Italy, but gradually moved to northwestern Europe, where it changed into an intensive form of aquaculture after the Japanese example.

As artificial reproduction of freshwater eel is not yet possible on a commercial scale, production for consumption is still based entirely on catches of wild glass eels or elvers. This leads to the potential introduction of eel disease agents in aquaculture production systems. *Anguilla anguilla* is nowadays produced generally in intensive recirculation systems at a regulated water temperature. With an annual production of about 4000 t in the previous decade, the Netherlands is the most important eel-producing country in Europe (FAO 2012). High stocking densities make detection and control of diseases vital for sustainable farming. Prevention and treatment of eel viral diseases is particularly difficult, as commercial vaccines are not available.

When high-density Japanese eel pond culture exponentially grew in the late 1960s and early 1970s in Japan, *Anguilla anguilla* and American glass eels and elvers *A. rostrata* were imported and stocked, and catastrophic viral disease outbreaks occurred frequently (Heinsbroek 1991). Permanently growing cell lines were developed from *A. japonica* kidney and ovary cells, and used for virus isolation (Chen & Kou 1981, Chen et al. 1982). During the many outbreaks, new viruses were isolated and shown to be the causative agent (T. Sano 1976, T. Sano et al. 1977, M. Sano et al. 1990). Although initial descriptions were usually thorough and detailed, nomenclature was ambiguous. Hence, several virus isolates were initially presented as a new virus, and later demonstrated to be highly similar to an already described virus.

Identification of pathogenic eel viruses is further complicated by the non-pathognomonic clinical signs and gross pathology of these eel viral diseases. In addition, virus isolation from clinically healthy eels (Castric & Chastel 1980, Bucke 1981, Castric et al. 1984, Chen et al. 1985, Shchelkunov et al. 1989, Haenen et al. 2002), as well as double infections with different viruses (Ahne & Thomas 1985, Haenen et al. 2002, van Ginneken et al. 2004, Varvarigos et al. 2011), have been observed. Several diagnostic assays have been developed for the detection of eel viruses, more recently with a focus on molecular assays. In eel farming, identification of the causative agent can be used to take adequate quarantine and water temperature regulation measures in order to reduce clinical signs and losses.

In this literature review, we give an overview of the current knowledge on the aetiology, geographical distribution, clinical signs, mortality and gross pathology of pathogenic European eel viruses. In addition, we present a retrospective analysis of diagnostic data from the Dutch National Reference Laboratory (NRL) for Fish Diseases over the period 1990 to 2011, which provides a historical overview on the viruses isolated from wild and farmed *A. anguilla* in the Netherlands in the past 2 decades. The 3 viral agents that are observed most commonly in *A. anguilla* are the aquabirnavirus eel virus European (EVE), the rhabdovirus eel virus European X (EVEX) and the alloherpesvirus anguillid herpesvirus 1 (AngHV1).

EEL VIRUS EUROPEAN

Aetiology

Since 1969, serious outbreaks of a new disease, called branchionephritis or viral kidney disease of *Anguilla japonica*, occurred every winter when water temperatures were below 20°C in eel culture ponds in Japan (T. Sano et al. 1981). The aetiological agent was isolated for the first time from imported *A. anguilla* using the rainbow trout gonad cell line RTG-2 (Wolf & Quimby 1962) in 1973, and tentatively named eel virus European (EVE) (T. Sano 1976). EVE was subsequently also isolated from *A. japonica*, and River's postulates were fulfilled. The type of cytopathic effect (CPE) caused by EVE resembled the type of CPE caused by the aquabirnavirus infectious pancreatic necrosis virus (IPNV) (T. Sano 1976, T. Sano et al. 1981). Electron microscopy (EM) revealed that EVE virions were non-enveloped polyhedrons with a diameter of 68 to 77 nm, only present in the cytoplasm of infected cells. EVE also resembled IPNV in terms of biological properties, such as polypeptide composition and the bisegmented double-stranded RNA genome (Nishimura et al. 1981a, T. Sano et al. 1981, Hedrick et al. 1983a). Hence, EVE

is a tentative member of the genus *Aquabirnavirus* in the family *Birnaviridae*. Other names for EVE or IPNV of eel include eel virus (EV) [Berlin] (Schwanz-Pfitzner et al. 1984) and pillar cell necrosis virus (PCNV; Lee et al. 1999a, 2001).

Aquabirnaviruses form an antigenically diverse group of viruses, with the type species IPNV being the aetiological agent of an acute contagious systemic disease of several species of freshwater and marine fish, molluscs and crustaceans (Rodriguez Saint-Jean et al. 2003). Mortality caused by IPNV in salmonids is high in fry and fingerlings, but rare in older fish. Survivors of the epizootic disease may become lifelong carriers. Host specificity and cell tropism are determined by viral proteins encoded by the larger RNA segment A (M. Sano et al. 1992), and the occurrence of natural reassortment has recently been shown (Romero-Brey et al. 2009). Interspecies transmission has not yet been demonstrated, but it would explain the wide range of host species (Bandin & Dopazo 2011). Historically, aquabirnavirus isolates were grouped as 1 of the 3 major serotypes, designated Ab, Sp and VR-299 (Macdonald & Gower 1981).

Neutralisation tests confirmed the close relationship of EVE with IPNV (T. Sano 1976, T. Sano et al. 1981), later specified to IPNV type Ab (Hudson et al. 1981, Okamoto et al. 1983). EVE and IPNV type Ab were also found to be similar in polypeptide and RNA composition, and clearly distinguishable from IPNV strains VR-299 and Sp (T. Sano et al. 1981, Hedrick et al. 1983a). Using cross-neutralisation assays with almost 200 IPNV isolates, Hill & Way (1995) later proposed a new serological classification, consisting of serogroup A containing serotypes A1 to A9, and serogroup B containing the single serotype B1. The prevalence of the different serotypes is geographical, with the aquatic birnaviruses in the USA generally belonging to serotype A1, those in South America and Asia to serotypes A1 to A3, those in Europe to A2 to A5, and those in Canada to A6 to A9 (Blake et al. 2001). More recent phylogenetic analyses based on deduced amino acid sequences of the VP2 and VP5 genes of larger RNA segment A showed that Japanese and Taiwanese EVE strains group with IPNV strain Ab in genogroup 3 (Blake et al. 2001, Zhang & Suzuki 2004).

Geographical distribution

Anguilla anguilla elvers were first imported into Japan in 1968, after which the epizootics of branchionephritis started occurring in *A. japonica* (T. Sano et al. 1981). It was therefore suggested — and allegedly proven — that EVE entered Japan with the import of *A. anguilla* elvers (T. Sano et al. 1981, Hedrick et al. 1983a). EVE/IPNV type Ab and IPNV type VR-299 were later also isolated from various *A. japonica* farms in Taiwan (Chen et al. 1985, Hsu et al. 1989, 1993). EVE/PCNV from diseased *A. japonica* in Japan was serologically most similar to IPNV serotype Sp (Lee et al. 1999a), but genetically closer related to strain Ab (Lee et al. 2001).

In 1977, Castric & Chastel (1980) isolated an IPNV-like agent called B_6 from *Anguilla anguilla* elvers along the French Atlantic coast, and showed its relatedness to IPNV serotype Sp by serum neutralisation tests. EVE related to IPNV serotype Ab was repeatedly isolated from an eel farm in the UK (Bucke 1981, Hudson et al. 1981). IPNV type Ab or EVE was isolated from different populations of wild and farmed *A. japonica* in Taiwan (Hedrick et al. 1983b, Wu et al. 1987). Several viruses isolated from the blood (4 isolates) and gonads (1 isolate) of *A. anguilla* with stomatopapillomas in Germany were identified as IPNV subtype Ab by serum neutralisation tests (Ahne et al. 1987). The first of these viral isolates — isolated in 1968 — was tentatively named EV [Berlin], but later characterised as a birnavirus (Schwanz-Pfitzner et al. 1984). IPNV types Ab and Sp were isolated from pools of *A. anguilla* elvers and eels from Denmark, the UK and France (Jørgensen et al. 1994). J. Plumb (Auburn University) isolated EVE serotype Ab from *A. rostrata* (cited in McAllister & Owens 1995). Double infections of farmed *A. anguilla* with EVE and AngHV1 were reported in the Netherlands (Haenen et al. 2002) and Greece (Varvarigos et al. 2011), and double infections with EVE and EVEX were reported in Germany (Ahne & Thomsen 1985) and Italy (van Ginneken et al. 2004). Overall, EVE has been detected in *A. japonica* in Japan and Taiwan, in *A. anguilla* in Japan, France, the UK, Germany, Denmark, the Netherlands and Greece, and in *A. rostrata* in the USA.

Clinical signs and mortality

EVE has been isolated from apparently healthy and diseased eels. Moribund *Anguilla japonica* showed rigidity or spasm of the body, retracted abdomen, congestion of the anal fin, and occasionally diffuse congestion on the abdomen and gills (T. Sano 1976, T. Sano et al. 1981). Wu et al. (1987) isolated an EVE-like virus from farmed *A. japonica* showing some pathological symptoms such as ulcer-

ative lesions over the body, congestion of the fins, atrophy of the muscles and a deformed trunk. Bucke (1981) and Hudson et al. (1981), however, isolated EVE from *A. anguilla* with no external lesions or abnormalities. Chen et al. (1985) isolated EVE from healthy and diseased *A. japonica*, and during an outbreak of branchionephritis with nearly 100% mortality in certain ponds. EVE/PCNV was isolated from mass mortalities among farmed *A. japonica* since the late 1980s, in which the eels showed no other external pathological signs, except for loss of appetite and general weakness (Lee et al. 1999a). In short, the most commonly reported clinical signs of EVE-infection in eel are an abnormal shape of the trunk, and congestion of the skin, fins and gills.

Gross pathology

Gross internal findings in *Anguilla japonica* and *A. anguilla* were some enlargement of the kidney, an empty gut, and in some cases ascites (T. Sano 1976, T. Sano et al. 1981). Histopathological findings included tubular and renal interstitial necrosis in the kidney, and occasionally focal necrosis in the liver and spleen. Wu et al. (1987) found hypertrophy and necrosis of the liver in *A. japonica*, while Hudson et al. (1981) occasionally found petechial haemorrhages in the liver of *A. anguilla*. The *A. japonica* from which Ueno et al. (1984) isolated a birnavirus similar to EVE had nephroblastoma, clinically manifested as whitish, swollen and solid kidneys. EVE/PCNV caused gill disease, characterised by aneurysmal haematoma formations in the gill lamellae and necrosis of the pillar cells (Lee et al. 1999a). Although EVE was isolated repeatedly from *A. anguilla* with stomatopapillomas, attempts to initiate tumour production in healthy eels by inoculation with this virus failed, suggesting no causative relationship (McAllister et al. 1977). Taken together, the most common gross pathological findings of EVE infection in eel are enlargement of the kidneys, necrosis or petechial haemorrhages in the liver, and gill disease.

Experimental infections

Experimental infections of eel and rainbow trout with EVE have yielded variable results. T. Sano (1976) tested the infectivity of EVE for *Anguilla japonica* glass eels and young eels experimentally by bath immersion and by intraperitoneal injection, respectively. Cumulative mortality over a 20 d period was 60% for the glass eels, which were held at 15 to 20°C (T. Sano 1976), and 55 to 75% for the young eels, which were held at 8 to 14°C (T. Sano 1976, T. Sano et al. 1981). Moribund young eels showed muscular spasm or rigidity, slight petechiae of the abdominal skin and congestion of the anal fin. EVE was reisolated from gill, spleen, gut and kidney tissue of the moribund young eels, and from whole glass eels. In a subsequent infection trial of *A. japonica* with EVE and with IPNV strain d'Honnicthun, no mortality occurred (T. Sano et al. 1981). It was possible to reisolate EVE, but not IPNV, from the injected eels. No significant mortalities were observed in *A. anguilla* elvers (0.25 and 0.50 g) infected with EVE/IPNV strain B_6 by bath immersion or sprinkling at 8–11 and 21.5°C, or in young *A. anguilla* (3 g) infected with EVE/IPNV strain B_6 by intraperitoneal injection at 17°C (Castric & Chastel 1980). The Ab serotype of EVE isolated from farmed *A. anguilla* from the UK did not cause disease in eels either (Hudson et al. 1981). The EVE-like virus isolated by Ueno et al. (1984) caused 40% mortality in juvenile *A. japonica* after intraperitoneal injection at 20 to 25°C. EVE/PCNV caused about 70% mortality in *A. japonica* (~40 g) during a 21 d experiment at 25°C, after intramuscular injection (Lee et al. 1999a). All inoculated eels showed aneurysmal haematoma formation in gill lamellae and stasis of gill filamental arteries, similar to natural infections, and it was possible to reisolate the virus from diseased gill filaments. Larger eels (~120 g) showed only limited gill disease and no significant mortality.

Castric & Chastel (1980) showed the pathogenicity of EVE/IPNV strain B_6 for rainbow trout *Oncorhynchus mykiss* fry, by bath immersion at 8 to 11°C, reaching cumulative mortalities of 82% over a 2 mo period. Affected fish showed typical signs of infectious pancreatic necrosis (IPN), and strain B_6 was easily reisolated from dead fry. However, T. Sano et al. (1981) did not find any significant signs or mortality in rainbow trout fry exposed to EVE by bath immersion at 10°C over a 40 d period. The Ab serotypes of EVE isolated from farmed *Anguilla anguilla* and *A. japonica* from the UK and Japan, respectively, did not cause disease in rainbow trout fry either (Hudson et al. 1981, Okamoto et al. 1983, M. Sano et al. 1992). The EVE isolate serotype Ab from *A. rostrata* appeared to be non-virulent to brook trout *Salvelinus fontinalis* fry 42 d old at 12°C; a Japanese EVE isolate was weakly virulent (3% virus associated mortality), and another Japanese EVE isolate was highly virulent (87% virus-associated mortality) (McAllister & Owens 1995). The EVE-like virus isolated by Ueno et

al. (1984) resulted in 25 to 35% mortality in intraperitoneally injected juvenile common carp *Cyprinus carpio* and hybrid tilapia at 20 to 25°C. An infection trial with tilapia at 10 to 16°C resulted in a cumulative mortality of 80% (Ueno et al. 1984).

In conclusion, outcomes of infection trials with EVE in elvers, young eels and rainbow trout fry vary. EVE seems to be pathogenic for rainbow trout fry, but not always for juvenile eels. The observed differences are most likely due to varying experimental conditions, such as age of the experimental fish, EVE strain used, infection method, and water temperature.

EEL VIRUS AMERICAN AND EEL VIRUS EUROPEAN X

Aetiology

In the 1970s, 2 rhabdoviruses were isolated from imported eel in Japan. The first was isolated from young *Anguilla rostrata* imported from Cuba in 1974 and designated eel virus American (EVA) (T. Sano 1976). A second related rhabdovirus was isolated 2 yr later from *A. anguilla* elvers imported from France and designated eel virus European X (EVEX) (T. Sano et al. 1977). EVA and EVEX are morphologically (enveloped bullet-shaped particles of 136–160 × 53–84 nm), serologically, physicochemically and genetically (single-stranded RNA genome) highly similar, and regarded as 2 strains of a single virus species (Hill et al. 1980, Nishimura et al. 1981b, van Beurden et al. 2011). Synonymous names used for EVEX/EVA were rhabdovirus anguilla (Hill et al. 1980, Shchelkunov et al. 1989) and rhabdoviral dermatitis of Japanese eel (Kobayashi & Miyazaki 1996). EVEX/EVA belongs to the group of fish vesiculovirus-like isolates, family *Rhabdoviridae*, order *Mononegavirales* (Galinier et al. 2012).

Geographical distribution

The first detection of EVEX in Europe originates from the early 1980s, when the EVEX-like viruses C_{30}, B_{44} and D_{13} were isolated during a virological survey on the wild *Anguilla anguilla* elver population of the Loire estuary along the French Atlantic coast (Castric & Chastel 1980, Castric et al. 1984). EVEX was subsequently isolated from *A. anguilla* from Germany (Ahne & Thomsen 1985, Ahne et al. 1987), and from *A. anguilla* elvers imported from Germany to Russia (Shchelkunov et al. 1989). In a comprehensive study on the occurrence of virus infections in pools of elvers and eels in Europe from 1977 to 1992, Jørgensen et al. (1994) isolated EVEX in *A. anguilla* from France, the UK, Denmark and Sweden. Van Ginneken et al. (2004) detected EVEX in wild eels originating from various geographic regions. In retrospective sequence analyses of these samples, however, only a single virus strain was observed, most likely having an EVEX-infected eel farm in Italy as its source (M. Y. Engelsma et al. unpubl. data). In the Netherlands, EVEX was later isolated from wild *A. anguilla* after a swim tunnel experiment (van Ginneken et al. 2005), and from various *A. anguilla* farms (Haenen et al. 2002, van Beurden et al. 2011). In Japan, a rhabdovirus causing dermatitis in *A. japonica* was isolated and shown to be serologically similar to EVEX and EVA (Kobayashi & Miyazaki 1996). Overall, EVA has been isolated from *A. rostrata* imported from Cuba, EVEX has been detected in *A. anguilla* from France, Germany, the UK, Denmark, Sweden, Italy and the Netherlands, and rhabdoviral dermatitis has been diagnosed in *A. japonica* in Japan.

Clinical signs and mortality

In the initial description of EVA by T. Sano (1976), the infected *Anguilla rostrata* showed clear external symptoms: most eels had a tendency to bend the head down, and showed intense congestion in the pectoral and anal fin and diffuse congestion over the abdominal skin. The American eels showed an unusually high mortality of 59% over a 170 d rearing period. *A. japonica* infected with rhabdoviral dermatitis during post-harvest stocking showed cutaneous erosion and ulceration (Kobayashi & Miyazaki 1996). This disease occasionally occurred and caused mass mortalities during the pre-shipping stocking.

EVEX-infected farmed *Anguilla anguilla* from Italy showed clinical signs such as haemorrhages and red skin areas (van Ginneken et al. 2004). However, on several occasions, EVEX has also been isolated from apparently healthy *A. anguilla* elvers (Castric & Chastel 1980, Castric et al. 1984, Shchelkunov et al. 1989). Although Ahne et al. (1987) repeatedly isolated EVEX from *A. anguilla* with stomatopapilloma, they did not think there was a causative relationship.

Gross pathology

Internal gross pathology findings specific for naturally EVA- or EVEX-infected eels have never been

recorded. Histopathological examination of EVA-infected *Anguilla rostrata* revealed intense haemorrhages and degeneration in the skeletal muscles, hyperaemia of the branchial vessels and haemorrhages in the Bowman's space and tubuli (T. Sano 1976).

Experimental infections

Several infection trials with EVA and EVEX in eel and other fish species have been performed. Nishimura et al. (1981b) tested the pathogenicity of EVA in carp and rainbow trout, and the pathogenicity of EVEX in *Anguilla japonica*, ayu *Plecoglossus altivelis*, carp and rainbow trout. Mortality and positive virus reisolation was only observed in EVA and EVEX bath-infected rainbow trout fry (0.2 to 0.3 g). Diseased trout became dark in colour, lost their appetite, became apathetic, gathered at the bottom of the aquarium and died quickly. Internally, gross haemorrhages in the kidney were most noticeable. Gross pathological and histopathological findings were very similar to that caused by infectious hematopoietic necrosis virus (IHNV). Mortality increased with the water temperature, being lowest at 10°C and highest at 20°C. In general, EVEX seemed to be more virulent (cumulative mortality was nearly 100% at 20°C) than EVA (cumulative mortality was 26% at 20°C). EVA and EVEX were reisolated from most of the dead trout, and from some of the surviving trout. Hill et al. (1980) and Hill & Williams (1984) confirmed that EVA and EVEX caused mortality in rainbow trout fry, and that the clinical symptoms were indistinguishable from those caused by viral hemorrhagic septicemia virus (VHSV). However, Castric & Chastel (1980) were not able to reproduce these results in several infection trials with EVEX and eel rhabdoviruses B_{12} and C_{30} in rainbow trout fry, and *A. anguilla* elvers and young eels.

Shchelkunov et al. (1989) injected EVEX intraperitoneally in 4 yr old *Anguilla anguilla* kept at 10.5 to 13.5°C, which resulted repeatedly in signs of haemorrhages in the interradial tissue of the fins, on the mucous membrane of the mouth and in the eyeball, exudate in the peritoneal cavity, oedema and anaemia of internal organs, and mortality rates up to 37.5%. Their EVEX isolate — originating from imported eel from Germany — did not appear to be pathogenic for yearlings of common carp and rainbow trout (I. S. Shchelkunov pers. comm.).

Intracutaneous injection of the EVA/EVEX-like virus causing rhabdoviral dermatitis in *Anguilla japonica* resulted in extensive cutaneous erosive lesions with haemorrhage, and histopathological findings similar to naturally infected eels (Kobayashi & Miyazaki 1996). The rhabdovirus resulted in 25 to 50% mortality at 15°C, 25% mortality at 20°C at the highest infective dose, and no mortality at 25°C (Kobayashi et al. 1999). The virus was reisolated from moribund and surviving fish.

In a swim tunnel experiment simulating the migration of *Anguilla anguilla* to the Sargasso Sea, EVEX-infected silver eels developed petechial haemorrhages all over the body, bloody abdominal fluid and anaemia, and died after swimming only 1000 to 1500 km (van Ginneken et al. 2005). Since the virus-negative animals were able to swim 5500 km successfully — the estimated distance from Europe to the Sargasso Sea — it was hypothesised that EVEX infection might impair the eels' natural spawning migration.

In conclusion, EVEX has experimentally been shown to be pathogenic for *Anguilla anguilla* and *A. japonica*, causing external haemorrhages, anaemia and mortality up to 50%.

EVEX and EVA have also been found to be pathogenic to rainbow trout fry, causing internal haemorrhages and mortality up to 100%. These results could not be confirmed by Castric & Chastel (1980), however.

ANGUILLID HERPESVIRUS 1

Aetiology

In 1985, herpesvirus-like particles were observed by EM in skin lesions of wild-caught *Anguilla anguilla* reared in a raceway system in Hungary at water temperatures of 26 to 28°C (Békési et al. 1986). The eels showed several skin lesions associated with mortality. The observed herpesvirus could not be isolated, however. In the same year, undefined mortalities were observed in *A. japonica* and *A. anguilla* farmed in recirculation systems at 30°C in Japan (M. Sano et al. 1990). The causative virus was isolated successfully in the eel kidney cell line EK-1 (Chen et al. 1982). Using EM, virions were shown to be composed of an icosahedral nucleocapsid (triangulation number or $T = 16$) with a mean diameter of 110 nm made up of hollow capsomers, a surrounding tegument, and an envelope with a diameter ranging from 185 to 210 nm, including spikes, i.e. the typical morphology of a herpesvirus. The isolated eel herpesvirus was tentatively named herpesvirus anguillae (M. Sano et al. 1990) and later designated anguillid her-

pesvirus 1 (AngHV1). Synonymous names include eel herpesvirus in Formosa (EHVF; Ueno et al. 1992, 1996), gill herpesvirus of eel (Lee et al. 1999b) and European eel herpesvirus (Chang et al. 2002). AngHV1 isolates from *A. anguilla* in Europe and Asia are serologically and molecularly highly similar, and can be considered as a single virus species (Chang et al. 2002, Rijsewijk et al. 2005, Waltzek et al. 2009). AngHV1 belongs to the genus *Cyprinivirus*, in the family *Alloherpesviridae* of the order *Herpesvirales* (van Beurden et al. 2010, ICTV 2012).

Geographical distribution

After the first isolation in Japan, several herpesviruses were isolated from *Anguilla japonica* in East Asia. From 1988 to 1990, a herpesvirus was isolated from diseased *A. japonica* in Taiwan, which was designated EHVF (Ueno et al. 1992). In a subsequent study, EHVF was shown to be highly similar to AngHV1 in cross-neutralisation tests, structural protein analysis and Western blot (Ueno et al. 1996). In 1992, a herpesvirus was isolated from *A. japonica*, reared in warm water ponds, showing erosive and ulcerative cutaneous lesions (Kobayashi & Miyazaki 1997). From 1993 to 1995, a herpesviral gill disease accompanied by mass mortality occurred in *A. japonica* reared in warm water ponds in Japan (Lee et al. 1999b). The virus was designated gill herpesvirus of eel, but was identified as AngHV1 by virus neutralisation. The presence of AngHV1 DNA was demonstrated in asymptomatic farmed *A. japonica* and *A. rostrata* in Taiwan by PCR (Shih 2004).

In Europe, several uncharacterised herpesviruses were isolated from apparently healthy wild and farmed *Anguilla anguilla* from France (Jørgensen et al. 1994). In 1998, a herpesvirus was isolated from diseased farmed *A. anguilla* in the Netherlands, and antigenically identified as AngHV1 (Davidse et al. 1999). Another herpesvirus, isolated from *A. anguilla* in Taiwan, was named European eel herpesvirus and shown to be genetically highly similar to AngHV1 (Chang et al. 2002). AngHV1 was subsequently isolated from wild *A. anguilla* from the Netherlands (van Ginneken et al. 2004, Haenen et al. 2010) and Germany (only PCR-positive) (Jakob et al. 2009), and from farmed *A. anguilla* from the Netherlands (Haenen et al. 2002, van Ginneken et al. 2004) and Greece (Varvarigos et al. 2011). The Dutch AngHV1 isolates were shown to be antigenically and genetically related to the Japanese AngHV1 isolate (Davidse et al. 1999, van Nieuwstadt et al. 2001, Rijsewijk et al. 2005). Overall, AngHV1 has been reported in *A. japonica* and *A. anguilla* in Japan and Taiwan, *A. anguilla* in the Netherlands, Germany and Greece, and *A. rostrata* in Taiwan.

Clinical signs and mortality

Clinical and pathological findings of AngHV1 infections varied among and within outbreaks, and were generally stress-induced (Chang et al. 2002, Haenen et al. 2002). Morbidity was high in some outbreaks (Davidse et al. 1999), and observed mortalities ranged from almost 0 up to 30% (M. Sano et al. 1990, Davidse et al. 1999, Chang et al. 2002, Haenen et al. 2002).

With regard to behavioural changes, apathy (Haenen et al. 2002) and a loss of appetite (Lee et al. 1999b) were recorded. The skin of affected eels showed varying degrees of erythema (M. Sano et al. 1990, van Ginneken et al. 2004), petechial and non-petechial haemorrhages (Davidse et al. 1999, Lee et al. 1999b, Haenen et al. 2002, van Ginneken et al. 2004), erosive and ulcerative lesions (Kobayashi & Miyazaki 1997, Davidse et al. 1999, Haenen et al. 2002), and varicella (Ueno et al. 1992), sometimes with a patchy appearance (Davidse et al. 1999, Haenen et al. 2002) or increased mucus secretion (Chang et al. 2002). The most affected regions included the head, mouth (Davidse et al. 1999), operculum (Davidse et al. 1999, Lee et al. 1999b), abdominal body surface (Lee et al. 1999b, Chang et al. 2002), and anal and urogenital region (Chang et al. 2002). The fins also showed haemorrhages (Davidse et al. 1999, Lee et al. 1999b, Haenen et al. 2002) and sometimes ulcerative lesions (Haenen et al. 2002) or bloody congestion of the anal fin (Chang et al. 2002). Although Haenen et al. (2010) observed fin haemorrhages in 72% of AngHV1-infected *Anguilla anguilla* silver eels, this clinical sign was found to be nonspecific.

Chang et al. (2002) found that affected eels only showed pale and swollen gills. However, most other studies reported more severe pathological changes, such as increased mucus secretion (Ueno et al. 1992, Chang et al. 2002), varying degrees of erythema (M. Sano et al. 1990), haemorrhages (Ueno et al. 1992, Davidse et al. 1999, Lee et al. 1999b, Haenen et al. 2002), partial fusion of the branchial lamellae resulting in mild necrosis (M. Sano et al. 1990), destruction of the filament tips (Lee et al. 1999b), and congestion (Lee et al. 1999b, Haenen et al. 2002). Overall, the most common clinical findings include apathy, varying degrees of skin and fin haemorrhages, and congestion of the gills.

Gross pathology

In natural AngHV1 infections, the internal findings ranged from clinically normal (Chang et al. 2002) to severely affected organs. The most apparent internal findings included paleness of the liver (Ueno et al. 1992, Davidse et al. 1999, Haenen et al. 2002), multifocal haemorrhages in the liver (Ueno et al. 1992, Davidse et al. 1999, Haenen et al. 2002), swelling of the kidney (Ueno et al. 1992, Lee et al. 1999b), and distension of the gall bladder (Ueno et al. 1992, Haenen et al. 2002). Less frequent findings include hepatic congestion (Lee et al. 1999b), marked enteritis (Ueno et al. 1992), an enlarged spleen (Davidse et al. 1999), pink fat caused by small diffuse haemorrhages (Davidse et al. 1999) and ascites (Haenen et al. 2002).

Experimental infections

Several experimental infections with AngHV1 have been performed and described, but severe disease was not generally induced. Ueno et al. (1992) injected *Anguilla japonica* (~112 g) and common carp (~15.2 g) intraperitoneally with AngHV1/EHVF, and observed the fish for 60 d at a water temperature of 10 to 19°C. Infected fish showed only increased mucus secretion on the gills, but no skin haemorrhages. Internally, the liver seemed to be slightly paler than normal. The eels showed no mortality, but AngHV1 was reisolated from the liver and kidney from all infected fish. The carp showed 37% mortality, and the virus was reisolated from all of the dead and the majority of the surviving fish. Similarly, Shih et al. (2003) did not observe any pathological changes until 7 wk after intraperitoneal infection with AngHV1 of *A. japonica* (~14.9 g) kept at 25°C.

Kobayashi & Miyazaki (1997) injected *Anguilla japonica* intracutaneously. The experimental infection did not result in any mortality after 14 d at 25°C, and cutaneous lesions — histologically similar to naturally infected *A. japonica* — were only observed at the site of injection.

Lee et al. (1999b) infected smaller *Anguilla japonica* (25 to 40 g) with AngHV1/gill herpesvirus of eel by intramuscular injection, and larger *A. japonica* (130 g) by gill arch injection. After 5 to 10 d at 25°C, some smaller eels showed skin haemorrhages at the site of injection and some haemorrhages in the gills. The virus was reisolated from the gills and kidneys from some moribund smaller eels. In the larger eels infected by gill arch injection, after 21 d, slight necrotic lesions were observed in gill filaments, but AngHV1 was not reisolated.

Hangalapura et al. (2007) infected post-larval *Anguilla anguilla* (~5.1 g) by bath immersion with AngHV1. During the 21 d rearing period at 24°C, 15% of the inoculated eels showed clinical signs, such as haemorrhages extending from the lower jaw, throat, operculum and pectoral fins, ventrally down to the tail. Virus infection was monitored by clinical signs, PCR, virus isolation, histopathology and immunohistochemistry, which all showed good correlation.

Van Nieuwstadt et al. (2001) experimentally demonstrated persistence of AngHV1 infection in farmed *A. anguilla*. Outwardly healthy and virus isolation-negative farmed *A. anguilla* (150 to 200 g) were shown to have antibodies specific for AngHV1. After being kept for several days at 23°C, some eels demonstrated either spontaneous or dexamethasone-provoked recrudescence of AngHV1, suggestive for the ability of AngHV1 to establish a latent infection.

In conclusion, experimental infection of *Anguilla japonica* and *A. anguilla* with AngHV1 resulted in a limited number of animals showing various degrees of external haemorrhages and pathology of the gills. AngHV1 did not cause any mortality in experimentally infected eels.

OTHER VIRUSES ISOLATED FROM *ANGUILLA ANGUILLA*

In addition to the 3 well-characterised eel viruses described above (EVE, EVEX, and AngHV1), several other viruses have been isolated from diseased *Anguilla anguilla* in the past. Discussed below are EV-1, EV-2, an orthomyxovirus-like isolate, and other rhabdoviruses isolated from *A. anguilla*. Descriptions of most of these isolates are limited, however, hampering proper taxonomic classification and assessment of their pathogenicity.

EV-1

In the early 1970s, Wolf & Quimby (1973) isolated a virus designated EV-1 from tumour and internal organ homogenates from *Anguilla anguilla* with stomatopapilloma originating from Germany. As EV-1 did not cause a lytic CPE in RTG-2 and fathead minnow (FHM; Gravell & Malsberger 1965) cells, but exhibited a CPE characterised by pyknotic, necrotic foci and massive syncytia, it was concluded that EV-1 was a virus other than EV [Berlin]/EVE (McAllister

et al. 1977). Using EM, small polyhedral particles were observed in the cytoplasm of infected cells. The relation of EV-1 to EV [Berlin]/EVE or the tumour is unknown, and no infection trials were performed. Another yet uncharacterised virus was isolated later from another tumour-bearing *A. anguilla* from Germany, and suggested to be similar to EV-1 based on its type of CPE (Ahne & Thomsen 1985).

EV-2 and another orthomyxovirus-like isolate from *Anguilla anguilla*

From the homogenates from which EV-1 was isolated, T. Nagabayashi and K. Wolf isolated another virus designated EV-2, causing a CPE characterised by diffuse foci of pyknotic cell masses and syncytia in FHM cells (McAllister et al. 1977, Nagabayashi & Wolf 1979). EM analysis revealed moderately pleomorphic 80–140 nm spheroid particles, possessing radially arranged 10 nm surface projections. By EM and indirect immunofluorescent microscopy, virus particles were observed only in the cytoplasm of infected cells, not in the nucleus. With the viral nucleic acid identified as RNA, the virus characteristics pointed in the direction of an orthomyxovirus-like agent. Intraperitoneal injection of EV-2 in North American elvers resulted in a cumulative mortality of 50% over a 3 mo period. However, virus could only be recovered from 25% of the moribund eels, and no significant histopathological changes were observed.

Another orthomyxovirus-like agent was isolated from wild-caught *Anguilla anguilla* elvers showing disease and high mortality (94.4%) directly after arrival at a Dutch eel farm (Munro et al. 2011). The elvers showed vertical swimming behaviour, loss of appetite, and yellow skin patches at the ventral body surface. Necropsy findings included pale gills with a congested epithelium, a pale liver and kidney, a congested gall bladder, haemorrhages in the spleen, and gas bubbles in the haemorrhagic intestine. The orthomyxovirus-like agent was isolated in the FHM cell line, and characterised by EM. Biochemical characterisation confirmed that the virus was an enveloped RNA virus.

Other rhabdoviruses isolated from *Anguilla anguilla*

When Castric et al. (1984) isolated 5 rhabdoviruses from *Anguilla anguilla* elvers from the Loire estuary, the 3 isolates C_{30}, B_{44} and D_{13} were serologically similar or closely related to EVEX, while 2 other isolates, B_6 and B_{12}, were classified as lyssaviruses—now tentative members of the genus *Novirhabdovirus*. The lyssavirus-like isolates failed to produce any mortality in *A. anguilla* elvers, young eel, and rainbow trout fry under various experimental conditions (Castric & Chastel 1980). Later, 3 more lyssavirus-like agents were isolated from pools of *A. anguilla* adults and elvers from France (Jørgensen et al. 1994).

A rhabdovirus isolate, L_{59X}, antigenically related to VHSV, was isolated from a pool of *Anguilla anguilla* elvers originating from the River Loire and several coastal rivers of Brittany, France (Castric et al. 1992). The virus appeared to be highly pathogenic for intraperitoneally infected rainbow trout fry (3 mo old) at 13°C, with a cumulative mortality of 89%, but much less pathogenic for 5 mo old rainbow trout fingerlings, with a mortality of only 15%. The pathogenicity of the isolated virus for eel, as well as the origin of the elver contamination, remains unknown.

In 1998, an IHNV isolate, DF13/98, was isolated from diseased farmed *Anguilla anguilla* kept at 23°C in Germany (Bergmann et al. 2003). Rainbow trout of various age and weight classes were infected with DF13/98 by immersion and reared for 28 d at 9°C. Fingerlings of 2.5 to 3 g did not show any clinical signs, but 28% of the fish died during the experiment. In larger rainbow trout of 15 to 20 g and 40 to 50 g, no symptoms or mortality were observed. IHNV was not reisolated from any of the infected fish at the end of the experiment. The pathogenicity of the new IHNV type DF13/98 for eel has not been studied yet.

VIRUSES ISOLATED FROM *ANGUILLA ANGUILLA* IN THE NETHERLANDS

The Dutch NRL for Fish Diseases is the only fully equipped diagnostic fish disease laboratory in the Netherlands. Since its establishment in 1985, it has regularly received batches of *Anguilla anguilla* glass eels and yellow eels from Dutch eel farms for clinical diagnostics or disease screening purposes. From 1998 onwards, wild *A. anguilla* yellow and silver eels from Dutch open water caught by fyke nets have been presented to the NRL as well. Data on all batches and individual eels tested for the presence of viruses over the period 1990 to 2011 are presented below.

Diagnostic procedures

Live *Anguilla anguilla* are transported to the Dutch NRL for Fish Diseases for diagnostic research. Upon

arrival, the eels are checked for clinical symptoms, anaesthetised, and euthanised by decapitation. The body cavity of each eel is opened and the internal organs are examined. Per eel, the spleen, kidney and liver are pooled for virus isolation, and from 1999 onwards, gills are collected separately. Applied materials and methods for cell culture and virus isolation have been published previously (Haenen et al. 2002). Briefly, 10% organ suspensions were prepared and filtered and unfiltered inoculated onto permissive cell lines at 15, 20 and 26°C. From 1990 to 1999, RTG-2 (Wolf & Quimby 1962) was used for virus isolation from eels, in which EVE, EVEX and several other uncharacterised eel viruses could be successfully isolated. From 1996 onwards, the EK-1 cell line (Chen et al. 1982) was used, in which AngHV1 could be additionally isolated. Two blind passages of 7 to 10 d were performed. After the appearance of CPE, the causative virus was determined by subsequent virus-specific assays. For EVE testing, an immunoperoxidase monolayer assay (IPMA) was developed (O. L. M. Haenen et al. unpubl. data). For EVEX testing, an indirect fluorescent antibody test (IFAT) was developed, and from 2008 on, real-time RT-PCR was used (van Beurden et al. 2011). For AngHV1 testing, from 1996 to 2005, an IPMA was used (Davidse et al. 1999), and from 2005 on, a PCR assay was used (Rijsewijk et al. 2005). If all 3 virus typing tests appeared to be negative, the uncharacterised virus was concentrated and characterised by EM as described previously (Haenen et al. 2002). If no CPE developed after 2 blind passages in cell culture, the 10% organ suspensions were considered virus-negative.

Viruses isolated from wild eels (1998–2011)

From 1998 to 2011, a total of 249 wild *Anguilla anguilla* eels from several rivers and lakes in the Netherlands were necropsied and tested for the presence of viruses (Table 1). Most samplings were carried out for monitoring purposes, while some samplings were initiated by disease outbreaks or unusual eel die-offs. Parts of this longitudinal study have been published elsewhere (van Ginneken et al. 2004, Haenen et al. 2010). *A. anguilla* yellow and silver eels were caught all year round on a total of 31 sampling occasions, with 21 sampling locations covering the most important eel habitats in the Netherlands (Fig. 1). In most cases, the pooled internal organs and gills of each eel were separately processed for virus isolation. In 14 cases, the organs of up to 10 eels were pooled.

A total of 36 eels and 5 pools of multiple eels were found to be virus-infected. The most commonly detected viruses were AngHV1 (10 occasions, 35 individual eels and 3 pools) and EVEX (1 pool), with 2 double infections of AngHV1 and EVEX (2 occasions, 1 individual eel and 1 pool). AngHV1 was isolated from eels from all over the Netherlands and during the entire monitoring period. EVEX was only found in 1998, 2001 and 2010 from 3 isolated locations.

Eels from 29 of the 31 sampling occasions showed varying degrees of clinical signs of disease (Table 1). The most common clinical findings were fin and skin haemorrhages and damage to the skin. Internally, many eels had an empty gut, most likely due to the capture by fyke net. About half of the eels showed pathological internal findings, most commonly a pale liver with multifocal haemorrhages. Twenty-five batches of eels showed light to severe infections with the swimbladder nematode *Anguillicoloides crassus*, and 15 batches of eels appeared to be infected with *Trypanosoma* spp.

Unusual mortality was reported in 5 cases. In the January 2001 case from a lake near Apeldoorn, several different fish species (including carp) showed

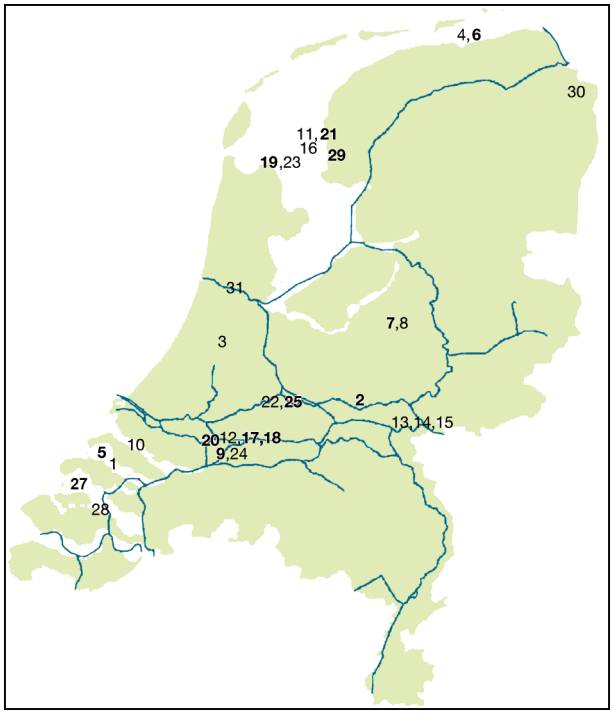

Fig. 1. *Anguilla anguilla*. Locations of 31 wild European eel sampling occasions (1998 to 2011) in the Netherlands. Numbers correspond with sample numbers in Table 1, with virus-infected sites shown in **bold**. Blue lines: main waterways

Table 1. *Anguilla anguilla*. Data on pathology and virus detection in European eels from several sampled rivers and lakes in the Netherlands (1998 to 2011) (see Fig. 1 for map). In the 'Clinical symptoms' and 'Gills' columns, light ectoparasitic infections of the skin and gills were considered normal and not mentioned. In the 'Internal parasites' column, *Trypanosoma* spp. were observed in fresh blood smears; intestinal worms were most often *Anguillicoloides crassus* larvae, sometimes *Acanthocephala* or cestodes. CVI: Central Veterinary Institute; *I. multifiliis*: *Ichthyophthirius multifiliis*; *A. crassus*: *Anguillicoloides crassus*; AngHV1: anguillid herpesvirus 1; EVEX: eel virus European X; nd: not done

Sample no.	CVI ref. no.	Date	Location	Dev. stage	Length (cm)	Clinical symptoms	Gills	Internal findings	Internal parasites	Virus-positive	Virus	Source
1[a]	488598	3-Mar-98	Dijkwater, Dreischor	Yellow eel	30–35	Apathy, petechiae and some skin and fin haemorrhages, dull eyes, congested skin	Haemorrhagic, some eels had mixed ectoparasitic infection[b]	Liver sometimes patchy appearance, distended gall bladder, bloody hindgut	30% *A. crassus*	Pool of 20	–	–
2[a]	509675	7-Sep-98	Rhenen	Yellow eel	65	Small lesions, petechiae on ventral body surface and anal fin, inflammation of anus	Haemorrhagic	Pale liver with some haemorrhages, mucous enteritis, pink fat	100% *A. crassus*	1 of 1	AngHV1 + EVEX	–
3[c]	531384	3-Jun-99	Lake Brasemer	Yellow eel	60–72	Darkening, some fin haemorrhages, *Trichodina* infection	–	–	20% *A. crassus* 100% *Trypanosoma* sp.	0 of 10	–	Van Ginneken et al. (2004)
4[c]	532663	17-Jun-99	Lake Lauwers	Yellow eel	58–70	Petechiae and lesions in skin and fins, mixed ectoparasitic infection[b]	Hyperplasia of epithelium, *I. multifiliis* infection	Some eels had patchy liver with petechiae; greenish mucous gut content	60% *A. crassus* 90% *Trypanosoma* sp.	0 of 10	–	Van Ginneken et al. (2004)
5[c]	534995	21-Jul-99	Lake Greveling	Yellow eel	~73	Small lesions in skin and fins	Some haemorrhages	Mucous gut content	–	1 of 10	AngHV1	Van Ginneken et al. (2004)
6[c]	535450	22-Jul-99	Lake Lauwers	Yellow eel	67–82	Lesions in skin, small haemorrhages in fins, *Ichthyobodo* infection	Hyperplasia of epithelium	Some eels had petechiae in liver	50% *A. crassus*	10 of 10	AngHV1	Van Ginneken et al. (2004)
7[a]	574856	16-Jan-01	Apeldoorn	Yellow eel	45–63	Apathy, enophthalmus	Hyperplasia of epithelium	Distended gall bladder	100% *Trypanosoma* sp.	Pool of 5	EVEX	–
8[a]	575779	31-Jan-01	Apeldoorn	Yellow eel	66	Apathy	Hyperplasia of epithelium	Patchy liver	–	0 of 1	–	–
9[a]	625534	4-Sep-03	Nieuwe Merwede	Yellow eel	37–75	Mechanical injury, reddening of tail, petechiae in skin, mouth and fins	Hyperplasia of epithelium, mixed ectoparasitic infection[b]	Some nonspecific findings	–	Pool of 17	AngHV1	–
10[c]	0402003 3/4	2-Nov-04	Haringvliet	Silver eel	53–83	Some eels had damaged skin, some eels had fin haemorrhages	–	Some nonspecific findings	67% *A. crassus* 17% *Trypanosoma* sp. 17% intestinal worms	0 of 6[d]	–	–
11[c]	0402007 2/3	11-Nov-04	Kornwerderzand	Silver eel	53–68	Some eels had damaged skin, some eels had fin haemorrhages	–	–	50% *A. crassus* 33% *Trypanosoma* sp. 33% intestinal worms	0 of 6[d]	–	Haenen et al. (2010)
12[c]	0402206 3/4	9-Dec-04	Boven Merwede	Silver eel	45–61	Some eels had damaged skin, some eels had fin haemorrhages	–	Some eels had enlarged spleen	50% *A. crassus* 17% *Trypanosoma* sp. 33% intestinal worms	0 of 6[d]	–	Haenen et al. (2010)

Table 1. (continued)

Sample no.	CVI ref. no.	Date	Location	Dev. stage	Length (cm)	Clinical symptoms	Gills	Internal findings	Internal parasites	Virus-positive	Virus	Source
13[c]	4022377	14-Dec-04	River Rhine	Yellow eel	80–81	Some skin damages and petechiae, heavy haemorrhages in the fins	–	Brownish blood	100% *A. crassus*	Pool of 2	–	–
14[c]	4022418	14-Dec-04	River Rhine	Yellow eel	62–75	Petechiae in skin and small haemorrhages in fins	–	–	25% *A. crassus*	Pool of 4	–	–
15[c]	4022419	14-Dec-04	River Rhine	Yellow eel	78–90	Small haemorrhages in fins	–	One eel had petechiae in muscles and mesenteria	100% *A. crassus*	Pool of 2	–	–
16[a]	5008242	12-May-05	Lake IJsselmeer	Yellow eel	28–35	Greenish skin, haemorrhagic head, blisters on head and along lateral line, dull eyes	Brownish, myxospore infection	Patchy orange liver	100% *A. crassus* 100% *Trypanosoma* sp. 100% intestinal worms	Pool of 3	–	–
17[c]	5015220	26-Aug-05	Beneden/Boven Merwede	Silver eel	43–85	Fin haemorrhages	*Trichodina* infection, some eels had *Dactylogyrus* infection	–	80% *A. crassus* 10% *Trypanosoma* sp. 50% intestinal worms	10 of 10[d]	AngHV1	Haenen et al. (2010)
18[a]	5016139	8-Sep-05	Boven Merwede	Yellow eel	62–67	Broken back, skinned, mechanical lesions, small fin haemorrhages	Congested (pale, brown) gills with gas bubble disease	Some nonspecific findings	*A. crassus* Intestinal worms	Pool of 10[d]	AngHV1	–
19[c]	5017527	28-Sep-05	Den Oever	Silver eel	52–84	Some small fin haemorrhages	–	Some eels had congested swimbladder	90%. *A. crassus* 40% *Trypanosoma* sp. 50% intestinal worms	3 of 10[d]	AngHV1	Haenen et al. (2010)
20[c]	5017879	5-Oct-05	Beneden Merwede	Silver eel	55–92	Some eels had damaged skin, fin haemorrhages	–	–	70% *A. crassus* 40% intestinal worms	8 of 10[d]	AngHV1	Haenen et al. (2010)
21[c]	5018319	12-Oct-05	Kornwerderzand	Silver eel	56–82	Fin haemorrhages	–	–	80% *A. crassus* 40% *Trypanosoma* sp. 60% intestinal worms	1 of 10[d]	AngHV1	Haenen et al. (2010)
22[c]	5018401	14-Oct-05	Lek	Silver eel	58–73	Fin haemorrhages	–	Some eels had congested swimbladder	90% *A. crassus* 20% *Trypanosoma* sp. 40% intestinal worms	0 of 10[d]	–	Haenen et al. (2010)
23[c]	5020305	10-Nov-05	Den Oever	Silver eel	57–84	Fin haemorrhages, some eels had damaged skin	–	–	80% *A. crassus* 40% *Trypanosoma* sp. 50% intestinal worms	0 of 10[d]	–	Haenen et al. (2010)
24[c]	5020752	16-Nov-05	Nieuwe Merwede	Silver eel	50–76	Fin haemorrhages, some eels had mechanical injury	–	–	60% *A. crassus* 30% *Trypanosoma* sp. 20% intestinal worms	0 of 10[d]	–	Haenen et al. (2010)

Table 1. (continued)

Sample no.	CVI ref. no.	Date	Location	Dev. stage	Length (cm)	Clinical symptoms	Gills	Internal findings	Internal parasites	Virus-positive	Virus	Source
25[c]	5021787	2-Dec-05	Lek	Silver eel	62–81	Some eels had fin haemorrhages	–	Some eels had haemorrhagic swimbladder	70% *A. crassus* 20% *Trypanosoma* sp. 60% intestinal worms	2 of 10[d]	AngHV1	Haenen et al. (2010)
26[a]	6029414	18-Oct-06	Southwest Drenthe	Yellow eel	40–50	Red pectoral fins	–	–	100% *A. crassus* 33% *Trypanosoma* sp. 100% intestinal worms	Pool of 3	–	–
27[a]	7022947	29-Aug-07	Oosterschelde	Yellow eel	~46	Skin lesions, haemorrhages in anal fin	–	Enlarged spleen, some nonspecific findings	–	Pool of 5	AngHV1	–
28[a]	7032848	18-Dec-07	Lake Veerse	Yellow eel	40–73	Apathy, large infected skin wounds, damaged tail, reddened anal fin	Pale and haemorrhagic gills	Dark spleen, enlarged pale liver with petechiae, pink fat and muscles, brown kidneys	50% *A. crassus* 50% intestinal worms	Pool of 4	–	–
29[a]	10013402	12-Aug-10	Workum	Yellow eel	28–60	Apathy, haemorrhages in mouth, skin and fins, damaged skin, red inflamed dorsal fin	–	Distended gall bladder	Some eels had *A. crassus* Some intestinal worms	Pool of 11	AngHV1 + EVEX	–
30[c]	10016086	30-Sep-10	Oldambtmeer	Yellow eel	40–56	–	nd	nd	nd	Pool of 10	–	–
31[c]	11010368	8-Jun-11	Noordzeekanaal	Yellow eel	32–42	–	*Trichodina* infection	–	Some eels had *A. crassus*	Pool of 13	–	–

[a]Sampling for diagnostic purposes because of disease outbreak or unusual eel die-off
[b]Double or triple infection with *Trichodina*, *Ichthyobodo*, *Dactylogyrus*, *Gyrodactylus*, or *Ichthyophthirius multifiliis*
[c]Sampling for monitoring purposes
[d]Virus isolation only performed at 20°C

mortality, which made a causative role of isolated EVEX unlikely. In the September 2003 and September 2005 cases from the rivers Nieuwe Merwede and Boven Merwede, respectively, AngHV1 was isolated from pools of silver *Anguilla anguilla*, but the cause of death was likely due to mechanical injury caused by hydroelectric power plant turbines in combination with a low water level during a hot summer. For the increased mortality rates of several fish species including *A. anguilla* in October 2006 from Southwest Drenthe, no infectious cause could be identified. In the August 2007 case, wild *A. anguilla* catches from the Oosterschelde estuary had declined by as much as 90%. The diseased eels showed bacterially infected skin wounds and AngHV1 was isolated.

Overall, pathogenic viruses were isolated from wild *Anguilla anguilla* from 12 different locations in the Netherlands during the past 14 yr. EVEX was only detected on 3 occasions, while AngHV1 was isolated 12 times. Clinical signs and pathological findings did not correlate with virus infection, and the effect of these pathogenic viruses on the local *A. anguilla* populations in the different areas is unclear.

Viruses isolated from glass eels (1990–2011)

From 1990 up to the present, *Anguilla anguilla* farmers from the Netherlands occasionally submitted glass eels intended for farming purposes to the Dutch NRL for Fish Diseases for clinical diagnostics. These wild-caught glass eels originated from different estuaries along the western European coast. Glass eels were either checked for the presence of pathogenic agents before stocking, or showed clinical signs of disease and/or increased mortality during the first weeks after arrival at the eel farms. Per batch, up to 10 glass eels were pooled, euthanised and ground in a mortar. Ten percent suspensions were then tested for the presence of pathogenic viruses by inoculation on permissive cell lines.

Over the 22 yr monitoring period, only 39 batches of glass eels were tested, making it difficult to detect trends in virus prevalence. More than half of the tested glass eel pools were not virus-infected (Fig. 2a). The most commonly detected pathogenic virus was AngHV1 (n = 10), and EVE, EVEX or an uncharacterised virus was found sporadically. Since the origin, transport routes and date of arrival at the farm were unknown in most cases, it is difficult to assess whether the wild-caught glass eels were already naturally virus-infected, or whether they became infected at the eel farm.

Viruses isolated from farmed eels (1990–2011)

From 1990 onwards, the majority of Dutch eel farmers (17 currently officially registered) regularly submitted live *Anguilla anguilla* to the Dutch NRL for Fish Diseases for diagnostics upon observing clinical signs or increased mortality rates, or for regular disease-screening purposes in the absence of clinical symptoms. In most cases, eels showed behavioural or clinical signs of disease, and/or increased mortality. Regularly, disease outbreaks were preceded by a stress trigger, such as a sudden change in water qual-

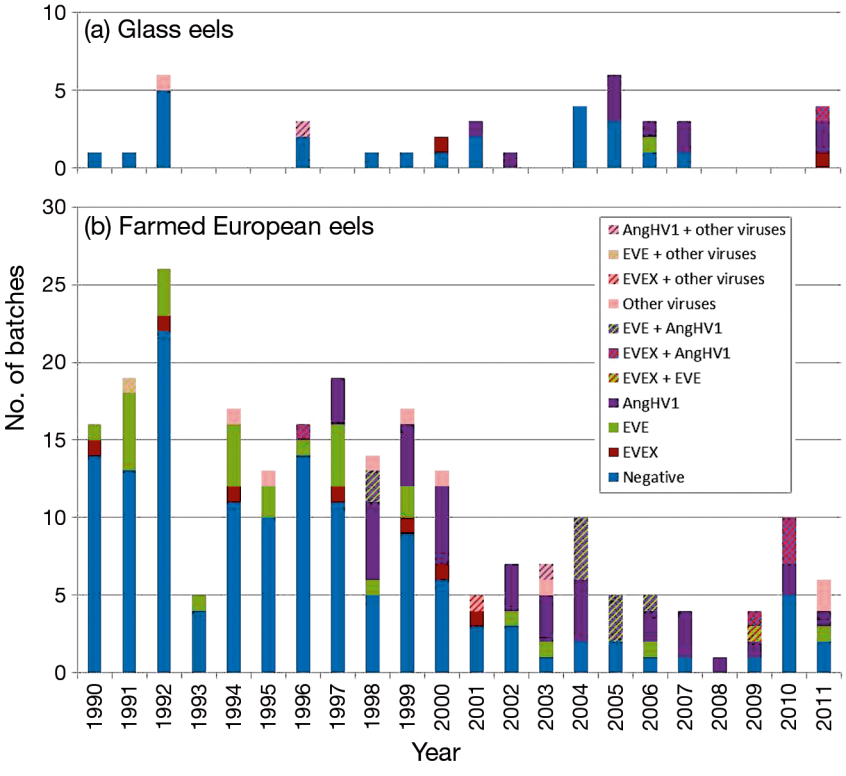

Fig. 2. *Anguilla anguilla*. Virus diagnostics at the Dutch National Reference Laboratory for Fish Diseases over the period 1990 to 2011 for (a) European glass eel (n = 39 batches) and (b) farmed European eel (n = 239 batches). AngHV1: anguillid herpesvirus 1; EVE: eel virus European; EVEX: eel virus European X; other viruses: all viruses other than AngHV1, EVE and EVEX

ity or size-sorting of the eels. In the case of farmed *A. anguilla*, internal organs and gills of up to 10 clinically diseased eels from the same system or farm — and not individual eels — were pooled and tested for the presence of pathogenic viruses as described above for wild eels (see 'Viruses isolated from wild eels (1998–2011)').

A total of 239 batches of farmed *Anguilla anguilla* were tested over a 22 yr period. More than half of the tested pools were negative for virus isolation (Fig. 2b). The most commonly isolated viruses were AngHV1 (n = 37), EVE (n = 28) and EVEX (n = 7). EVE and EVEX outbreaks mostly occurred at eel farms with water temperatures of 15 to 20°C, whereas AngHV1 generally caused disease at higher water temperatures (around 26°C). Double infections with 2 pathogenic viruses were regularly found, most commonly AngHV1 with EVE (n = 10) and AngHV1 with EVEX (n = 5). This phenomenon has serious implications for eel virus diagnostics, in which the presence of >1 virus species should always be considered. In addition, a double infection limits the possibility of controlling the disease outbreak by adjusting the water temperature to a non-permissive temperature for both viruses.

Occasionally, a yet uncharacterised virus was isolated and typed by EM; in most of these cases, a reovirus-like agent was found. In general, EVE was predominantly found from 1990 to 1997, while AngHV1 was predominantly found from 1997 to 2010. As AngHV1 does not propagate in the RTG-2 cell line, which was used until 1999, but can be isolated in the EK-1 cell line, which has been used since 1996, AngHV1 might have been present — but not detected — in samples collected and tested before 1996. In addition, some Dutch eel farmers immunise newly arrived elvers with AngHV1-infected water from the ongrow system, inducing a mild viral infection and supposedly protective immunisation (O. L. M. Haenen unpubl.). This may have biased the number of reported AngHV1 infections in farmed eels.

In most cases, the investigated *Anguilla anguilla* showed nonspecific clinical signs of disease, such as fin and skin haemorrhages, and sometimes local bacterial skin infections. EVE-infected farmed *A. anguilla* showed congestion of the skin, fins and gills, with severe fin haemorrhages, and anaemia. AngHV1 and EVEX infections were more often characterised by reddening of the fins and petechial skin haemorrhages, generally concentrated in the head region and ventral part of the body. Severely AngHV1-infected *A. anguilla* sometimes showed a typical tiger-like haemorrhagic pattern in the skin. Internally, various pathological findings were reported, with the liver being most commonly affected, characterised by paleness and multifocal smaller and larger haemorrhages.

In conclusion, the most commonly detected viruses from farmed *Anguilla anguilla* in the Netherlands were AngHV1, EVE and EVEX. Viral disease outbreaks were usually stress-triggered, temperature-dependent, and accompanied by secondary infections. Virus-infected eels usually showed clinical signs of disease, but clinical signs alone were not found to be a marker for virus infection.

FUTURE RESEARCH DIRECTIONS

Our current knowledge on pathogenic European eel viruses is hampered by a number of factors. First, there is a lack of peer-reviewed publications on the prevalence, clinical signs, mortality and gross pathology of these viruses, especially with regard to the wild *Anguilla anguilla* stock. Only a handful of studies have investigated the virological status of wild *A. anguilla* in Europe (Castric & Chastel 1980, Castric et al. 1984, Jørgensen et al. 1994, van Ginneken et al. 2004, Jakob et al. 2009, Haenen et al. 2010). As clinical signs and gross pathology were not recorded in most studies, the results only give an indication of the presence of particular viruses in the wild *A. anguilla* stock. The recent concern about the decline of the wild *A. anguilla* stock opens up new possibilities for studying the general health status of the wild *A. anguilla* population and the potential role of diseases in its decline (Haenen et al. 2012), which will support scientifically based restocking strategies.

Second, several of the eel viruses described early on are incompletely characterised. Many of these viruses were initially described as separate species, but are likely separate isolations of the same virus species. A solution might be found nowadays by sequencing analyses of the original isolates, such as EV [Berlin], EVE and EVA.

Third, the susceptibility of different freshwater eel species to the various virological agents is unknown. Many of the viruses presented in the present review were initially isolated in Japan, after shipment of *Anguilla anguilla* and *A. rostrata* for farming purposes. The identified viruses were subsequently isolated from Japanese eel too, and successful infection trials demonstrated the susceptibility of *A. japonica* and *A. anguilla* to similar viruses. With regard to *A. rostrata*, only 3 publications briefly report on the isolation or detection of pathogenic viruses (T. Sano

1976, McAllister & Owens 1995, Shih 2004). At least one virus has now been found in *A. japonica* and not in *A. anguilla* or *A. rostrata* (Mizutani et al. 2011). Concerning the introduction of new viruses via the import of foreign eel species, it is worth mentioning that *A. anguilla* is currently considered a critically endangered species by the IUCN (Freyhof & Kottelat 2010), and the export of live *A. anguilla* is hence restricted (CITES 2007).

CONCLUSIONS

The most commonly observed pathogenic viruses in *Anguilla anguilla* are AngHV1, EVE and EVEX. All 3 viruses may cause a haemorrhagic disease with increased mortality rates, but have been isolated from seemingly healthy eels too. In addition, latency has been suggested for AngHV1, and a carrier state for EVE. AngHV1 seems to be host-restricted to *A. japonica* and *A. anguilla*, while EVEX and EVE have also been shown to be able to cause disease in rainbow trout fry. In the Netherlands, AngHV1 has been regularly isolated from wild and farmed *A. anguilla*, EVEX sporadically, and EVE only from farmed eels. Viral disease in farmed eel is usually stress-triggered and temperature-dependent. Future research should focus on the genetic characterisation of historical isolates, the health status of the wild eel populations all over Europe, the potential role of diseases in the decline of the *A. anguilla* stock, and virus screening of farmed eel batches for restocking purposes into the wild.

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