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Monitoring of herpesvirus anguillae (AngHV-1) infections in the European eel in north-west Poland

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Summary

The European eel (Anguilla anguilla) is an economically important species in Poland, not only due to the quality and price of its meat, but also due to the eel stock management plan implemented since 2010. However, the imported juvenile eels for reintroduction are directly introduced into waters or reared in fish farms without necessary monitoring of the health status, especially for hazardous and pathogenic viruses. The study was conducted in the second half of 2014 using the European eels collected from Poland (Dąbie Lake, Szczecin Lagoon, hatcheries) and Denmark, mainly from importers and organizations producing material for reintroduction. A total of 256 samples were analysed including gills, liver, kidney, spleen, intestine and heart. Detection of the AngHV-1 was performed using pair primers designed to amplify the gene fragment encoding the viral DNA polymerase. Each PCR product was confirmed by bidirectional sequencing and sequence alignment with GenBank sequences. Obtained results indicate that the infection status of the eels imported to Poland is in the range of 0% to 100%, with the highest percentage of asymptomatic carriers among the fish imported from Denmark for the purpose of reintroduction and rearing. The eels collected in natural waters demonstrated an AngHV-1 infection rate of 30% (Dąbie Lake) and 40% (Trzebież). Bioinformatics analysis demonstrated that all these sequences were identical and represented the haplotype characterized for the first time in Poland. The intensity of eel reintroduction in Poland is 1.2 million individuals per year, which permits a conclusion that the species is important for the fishing economy and should be monitored against AngHV-1. The demonstrated existing high risk posed by mass kills of the European eel in domestic waters after the introduction of the herpesvirus AngHV-1 from imported fish.

Keywords: reintroduction programme, Anguilla anguilla, AngHV-1, imported fish, herpesvirus

The European eel (*Anguilla anguilla* L.) is an economically important species in Poland, not only due to the quality and price of its meat, but also due to the eel stock management plan implemented since 2010. The habitat of this species in Poland includes almost all types of waters; however, of major importance are transitional waters, mainly of the Vistula Lagoon and the Szczecin Lagoon, and lakes located in the northern part of the country (11). The average size of eel catches in the years 2009-2011 in the catchment areas of the Oder and Vistula rivers was 201.1 tonnes. However, in 2011, a downward trend in the catches to 170.4 tonnes was observed in both catchment areas. A similar phenomenon is observed in the case of eel catches in marine and coastal waters, in which the average value in 2009-2011 was 40.9 tonnes, with the lowest value of 31.2 tonnes obtained in 2011 (12). The intensity of eel reintroduction in Poland is 1.2 million individuals per year, which permits a conclusion that the species is important for the fishing economy. One of the main threats for fisheries and aquaculture are viruses, especially herpesviruses, which form a group of the most frequently occurring DNA viruses in fish (7). The virus was isolated in 1985 from ill European and Japanese eels, cultured in EK-1 and EO-2 cell lines and identified as *Herpesvirus anguillae* (AngHV-1) (14). The clinical signs of AngHV-1 infection are characterized by intense reddening of the head area, especially the mouth. Moreover, petechiae at the base of the fins, enlarged kidney and spleen, as were as necrosis of the skin, gills, liver and spleen have been observed (5). Neither in Poland nor in other EU countries (the Netherlands, Germany) is there an obligation to conduct tests for the detection of AngHV-1 (EU 2006/88/EC). This means that there is no legal barrier prohibiting the introduction of the European eel carrying identified AngHV-1 genome into open waters. In Poland imported material for reintroduction is directly introduced into waters or reared in fish farms without

virus monitoring. Therefore, the aim of the study was to verify the health status of the European eel distributed in north-west Poland to provide information on hazardous and pathogenic AngHV-1.

Material and methods

The study material consisted of 86 eels obtained in the second half of 2014 from importers and organizations producing material for reintroduction. The size of the eels ranged from 12 to 88 cm. The study was conducted using European eels collected from Polish fishermen using fyke nets in the waters of Dąbie Lake (n = 20) and the Szczecin Lagoon (n = 15). Other samples were juvenile eels imported for rearing from Denmark and purchased from a Polish importer (Dzwonowo, n = 6; Koszalin branch of Polish Angling Association (PZW), n = 31; and Stepnica, n = 9). Eels originating from the experimental fisheries station (RSD) Dolna Odra (n = 5) were intended for pre-rearing. After being transported to the rearing centre, they had contact with the cooling water from the Dolna Odra power plant in Nowe Czarnowo. The study was conducted in 2015 in the genetics laboratory of the Department of Aquaculture, West Pomeranian University of Technology, Szczecin, Poland.

From each of the 86 obtained individuals, gill slices were taken. As for the imported individuals, fragments of internal organs (liver, kidney, spleen, intestine and heart) were sampled, combined at an equal weight ratio and treated as a single sample (pool). A total of 256 samples were analysed. DNA was isolated from each sample using the peqGOLD Tissue DNA Mini Kit (PeqLab, Germany) and DNAzol Reagent (Invitrogen). The isolation of the nucleic acid was performed according to the instructions attached to the kits. The qualitative and quantitative assessment of the obtained DNA was carried out by electrophoresis of DNA isolates in 1.5% agarose gel followed by absorbance measurements using the NanoDrop 2000 UV-Vis spectrophotometer (Thermo Scientific). Detection of the gene fragment encoding the viral DNA polymerase AngHV-1 was conducted using the primers HVAPOLVPSD and HVAPOLOOSN, according to the procedure developed by Rijsewijk et al. (13) (Tab. 1). The results of each PCR were verified by separating the samples in 1.5% agarose gel followed by bi-directional Sanger sequencing of each PCR product (Genomed, Poland). The results of sequencing were analysed using BLAST, MEGA5 and BioEdit software (1, 6, 16).

Tab. 1. Primer pair used in the study to detect the AngHV-1 genome

Primer name	Primer sequence	Reference
HVAPOLVPSD	5'-GTG TCG GGC TTT GTG GTG C-3'	Rijsewijk et al. (13)
HVAPOLOOSN	5'-CAT GCC GGG AGT CTT TTT GAT-3'	

Tab. 2. Comparison of the number of samples containing AngHV-1 according to the method of DNA isolation

Collection site	Sample type	Number of AngHV-1-PCR-positive samples vs. total samples (% of PCR positive eels)	
		Column	DNAzol
Dzwonowo (n = 6)	Gills	0 (0%)	0 (0%)
	Pool	0 (0%)	0 (0%)
Dąbie Lake (n = 20)	Gills	6 (30%)	5 (25%)
Trzebież (n = 15)	Gills	6 (40%)	4 (27%)
Denmark (n = 9)	Gills	6 (66%)	4 (44%)
PZW Koszalin (n = 31)	Gills	29 (94%)	19 (61%)
	Pool	22 (71%)	15 (48%)
RSD Dolna Odra (n = 5)	Gills	5 (100%)	3 (60%)
	Pool	3 (60%)	1 (20%)
Positive samples/total number of samples		77/256 (30%)	51/256 (20%)

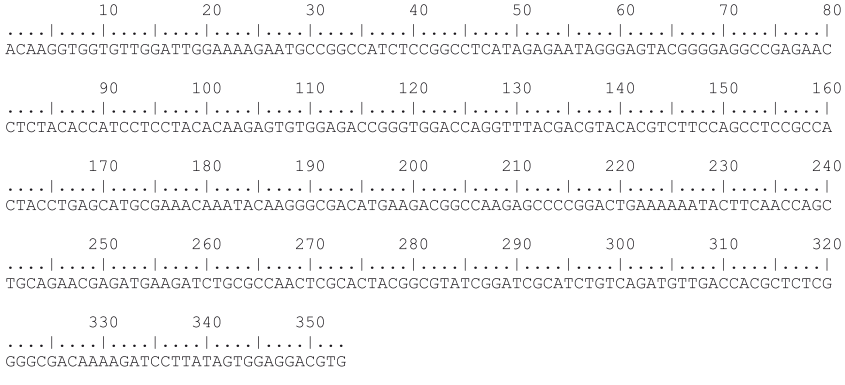

Fig. 1. Fragment of the AngHV-1 DNA polymerase gene sequence

Results and discussion

The qualitative and quantitative analysis of the obtained DNA isolates demonstrated that the column method provided isolates characterized by a higher degree of purity. The purity of DNA obtained using this method was in the correct range of 1.8-2.0, while that of DNA obtained using DNAzol was often below 1.8. As a consequence, differences in the number of positive samples obtained via PCR were observed. Overall, the amplification reactions yielded 30% and 20% of specific PCR products from the isolates obtained using the column method and DNAzol, respectively (Tab. 2). Bi-directional sequencing yielded sequences of 353 bp. Bioinformatics analysis demonstrated that all these sequences were identical and represented the haplotype characterized for the first time in Poland by Kempter et al. (9). The haplotype is the most common variant of a fragment of the AngHV-1 DNA polymerase gene. It is fully homologous with the sequences obtained from the analysis of eel samples from China or Denmark and GenBank records (e.g. AF333066, FJ940765) (3, 9, 13). The obtained fragment of the AngHV-1 DNA polymerase gene sequence is presented in Fig. 1. For unknown reasons, the number of glass eels migrating to the coasts of Europe is decreasing. In Germany, the decrease is estimated at 1/10, while in France, at 1/7 of the number observed in 1980s (15). In numerous European countries, eel stock management plans are implemented, including reintroduction of specified quantities of this species into waters. However, reintroduction plans pose a risk of introducing a pathogen that may potentially infect other eels. Such risk is

posed by herpesviruses which occur in both humans and animals. Herpesvirus infections are dangerous as they can occur in latent form or remain in the fish and in the environment for a long time after the recovery of the fish. This leads to a permanent presence of herpesviruses in the aquatic environment, but often also in bottom sediments or submerged plants. Much more dangerous is the presence of herpesviruses in invertebrates (10), due to the potential secondary transmission of the virus to the fish.

According to the results of this study, the problem of herpesvirus infection in eels due to the presence of AngHV-1 regards not only the fish that had contact with waters from rearing sites or natural waters in which they were pre-reared. The infection may originate from imported eels, thus the virus is introduced into the environment in which the eels may not have had previous contact with AngHV-1. In light of the fact that eel mortalities have been observed in Europe in the last 10 years and eel population has declined (5), the monitoring of the health of eels, with a special focus on viral diseases, should be a subject of particular attention. Eels imported to Poland, according to a report of 2012, are free of EVEX, AnHV-1 and other viruses pathogenic for the fish species produced in Polish aquaculture (12). However, as demonstrated in this study, the infection status of the eels imported to Poland is in the range of 0% to 100%, with the highest percentage of asymptomatic carriers among the fish imported from Denmark for the purpose of reintroduction (Koszalin branch of PZW) and rearing (RSD Dolna Odra). The eels collected in natural waters demonstrated an AngHV-1 infection rate of 30% (Dąbie Lake) and 40% (Trzebież). The cases of positive results indicate that the waters of one of the largest lakes in north-western Poland and the waters of the Szczecin Lagoon are not free of AngHV-1. Reintroduction of eels in the zones in which no fish infected with AngHV-1 have been found is highly contraindicated in such epizootic circumstances. In England, the presence of AngHV-1 in wild European eels was confirmed for the first time in 2009-2010 (2). In contrast to the situation in Poland, the death of eels occurred in waters at 17-19.4°C with symptoms typical of herpesvirus infections. According to the authors, detection of HVA in England is of very high importance in the implementation of a conservation and management programme focused on this species. It is also a determining factor in the implementation of reintroduction plans. An analysis of 140 eels from northern Germany (10) revealed the presence of AngHV-1 only in the eels from marine waters (Helgoland). The eels did not reveal any clinical signs, which confirms their carrier nature but, due to the geographical localization of their catch site, indicates the risk of spreading the virus in natural waters.

As has been shown in studies, AngHV-1 is disseminated in many countries across Europe, although its diagnostics is not coordinated in any way. Countries in which eel management programmes have been implemented, including Poland, should pay particular attention to the risk of transferring AngHV-1 into waters in which the virus has not arrived yet. It is not indicated to perform reintroduction with infected eels, and according to this study, an average of 30% of the investigated eels carried the virus. Due to the homogeneity of the analysed sequence with the sequence obtained in the first HVA detection in Poland (9), the virus probably does not mutate as frequently as, e.g., koi herpes virus. However, the genetic stability and affinity of the isolates do not decrease the risk associated with the species tropism of AngHV-1. The results presented in this study clearly indicate the risk posed by mass kills of the European eel in domestic waters after the introduction of the herpesvirus AngHV-1 from imported fish.

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