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CO-PRESENCE OF HERPES VIRUS ANGUILLAE (HVA) AND INFECTIOUS PANCREATIC NECROSIS (IPN) VIRUS IN EUROPEAN EELS *Anguilla anguilla*: FIRST DETECTION IN GREECE

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Background

In recent years (2008-2010) eel farming in the northwestern region of Greece has been severely affected by unexplained mortalities. Losses generally appeared after stressful conditions as handling or grading. Outbreaks were very frequent and were characterized by a slight increased daily mortality. Bacteriological and parasitological examinations, respectively, revealed the presence of *Aeromonas spp.* infections and *Dactylogyrus spp.*, *Trichodina spp.* and *Myxidium spp.* infestations, but these findings were not enough to explain the characteristics of these pathological outbreaks. Treatments with antibiotics and antiparasitic baths did not improve the situation. Necropsy displayed gills bleeding and inflammation, red sore-like dermal lesions around the head and the operculum, frequent hemorrhages in the pectoral fins. Such signs have focused the attention on a viral infection.

Methods

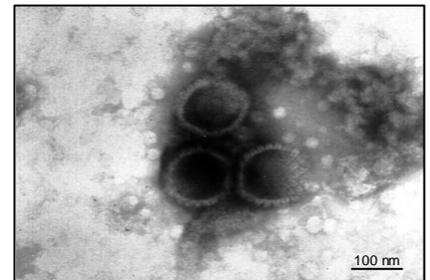
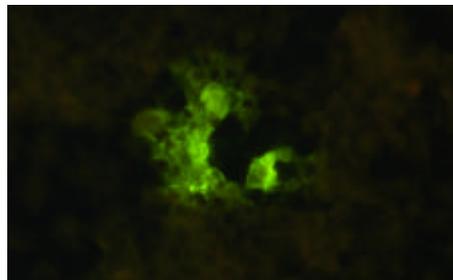
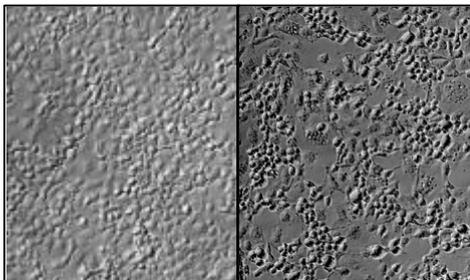
Frozen samples, collected in summer 2010, were tested for virological examinations. Virus isolation was performed on EPC, BF-2 and EK-1 (Chen & Kou 1982) cell culture monolayer. Virus identification was performed with IFAT with a polyclonal antibody raised in rabbits. Aliquots from tissue homogenates prepared for virus isolation were processed for TEM (Doane & Anderson 1987) investigations. Finally, a serotyping test of isolates with monoclonal antibody was performed.

Results

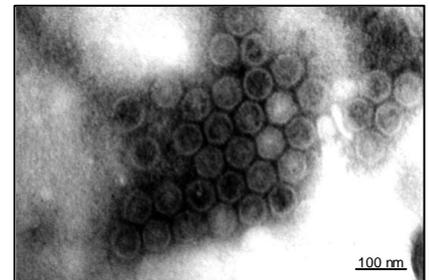
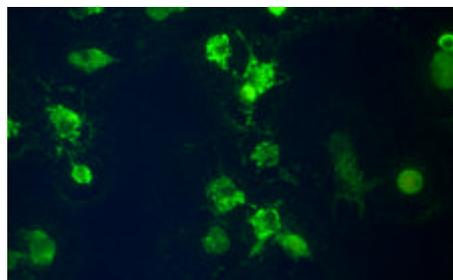
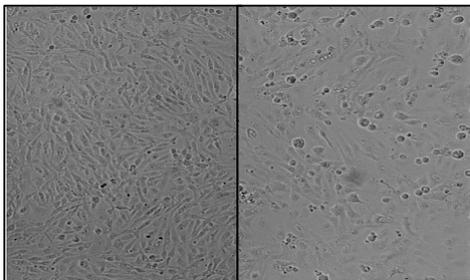
Clear CPE appeared on cell monolayer EK-1 infected with gill supernatant, as well as on BF-2 infected with supernatant obtained from pool of organs. Electron Microscopy detected the presence of both Birnavirus-like particles and Herpes-like particles. Immunofluorescence revealed the presence of HVA on EK-1 and IPNV on BF-2. IPN virus isolated was typed with monoclonal antibodies, which revealed an Sp serotype.



Hemorrhages and erosions in the lower jaw



On the left EK-1 monolayer, HVA cpe on EK-1 monolayer, positive IFAT staining for HVA in the middle and herpes-like particle observed by TEM on the right



On the left BF-2 monolayer, IPN cpe on BF-2 monolayer, positive IFAT staining for IPN in the middle and birnavirus-like particle observed by TEM on the right

Conclusion

In order to implement control measures and prevent re-infections of either HVA or IPN virus it is important to evaluate the magnitude of the diseases and their spread by specific surveillance programs in wild and farmed eel populations. Nevertheless, neither official control measures nor coordinated actions by the farmers are expected, hence, HVA and IPN could become in the future serious causes of economic loss in eel farming.

References

Chen SN and Kou GH (1982). A cell line derived from Japanese eel (*Anguilla japonica*) kidney. Proceedings of the National Science Council B, Republic of China 6, 93-100.
 Doane FW and Anderson N (1987). "Electron microscopy in diagnostic virology." Cambridge University Press, 178 p.

Acknowledgements

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