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WORKSHOP

Neglected viral diseases in freshwater fish farming

O. Haenen[1]*, L. Bigarré[2], T. Ito[3], J.-C. Avarre[4] and N. Vendramin[5]

[1]Corresponding author, Leading organizer, Wageningen Bioveterinary Research, NRL for Fish, Crustacean and Shellfish Diseases, Lelystad, The Netherlands; [2]ANSES, Plouzané, France; [3]Tamaki Laboratory, Aquatic Animal Health Division, National Research Institute of Aquaculture (NRIA), Fisheries Research Agency, Mie 519-0423 Japan; [4]ISEM, IRD, CNRS, EPHE, University of Montpellier, Montpellier, France; [5]Technical University of Denmark, National Institute of Aquatic Resources, Aquatic Animal Health group , 2800 Kgs. Lyngby, Denmark

Introduction

Worldwide, viral diseases pose a serious challenge to the fish farming industry and wild fish stocks. In order to prevent the spread of serious fish diseases and to contain and control fish disease outbreaks at a global scale, the World Organisation for Animal Health (OIE) has published relevant data on these fish diseases, both in the Aquatic Animal Health Code (OIE, 2017a), including the OIE listed fish diseases, and in the Manual of Diagnostic Tests for Aquatic Animals (OIE, 2017b), including recommended diagnostic methods. The list of viral OIE notifiable fish diseases currently includes: Epizootic haematopoietic necrosis disease (EHN), Infection with HPR-deleted or HPR0 infectious salmon anaemia virus (ISA), Infection with salmonid alphavirus, causing Pancreas disease (PD) and Sleeping disease (SD), Infectious haematopoietic necrosis (IHN), Koi herpesvirus disease (KHVD), Red sea bream iridoviral disease (RSIVD), Spring viraemia of carp (SVC), and Viral haemorrhagic septicaemia (VHS). At European Union level, Commission Decision 2006/88/EC and Council Implementing Decision 2015/1554/EC provide specific regulation for surveillance and control of listed infectious aquatic diseases in Europe which include the fish viral diseases VHS, IHN, EHN, ISA, and KHVD (European Commission 2006, 2015).

Due to the frequent emergence of new serious fish viral diseases, the OIE list of fish viral diseases is regularly being extended. However, apart from these well studied viral diseases, other non-notifiable serious fish viral diseases occur in freshwater fish farming. To focus on these new viral threats for freshwater fish farming, an open workshop was organised at the EAFP Conference in Belfast, 4th September 2017. The workshop consisted of five short lec-

* Corresponding author's and workshop leading organizer e-mail: olga.haenen@wur.nl

tures and a discussion, involving an audience of 69 international experts, originating from 25 countries, of which 6 were from outside Europe.

The main topics presented included issues related to:
1) the difficulty of preventing the global spread of cyprinid herpesvirus 2 (CyHV-2), 2) perhabdoviruses as a threat for percid farming, 3) pathogenesis and diagnostics of piscine orthoreoviruses in farmed rainbow trout, 4) Carp Edema Virus (CEV) in Europe, and 5) the potential role of fish endogenous retroviruses in disease emergence. The general aim of the workshop was to identify potential collaborative approaches to carry out multidisciplinary studies aiming to define risks, diagnostic methods and suggest adequate prophylactic measures.

Difficulty of preventing spread of Cyprinid Herpesvirus 2 (CYHV-2)

Takafumi Ito and Olga L. M. Haenen

Cyprinid herpesvirus 2 (CyHV-2) is known as the causative agent of herpesviral haematopoietic necrosis (HVHN) in goldfish (*Carassius auratus auratus*) and the occurrence of the virus has also been reported from multiple countries. CyHV-2 has caused death in goldfish regardless of the economic value. Moreover, the virus has also been detected from Prussian carp (*C. gibelio*) and crucian carp (*C. carassius*) from European and Asian countries.

In order to reduce economic losses to the aquaculture industry and impacts on natural resources by CyHV-2 infection, prevention of the spread and any further introductions of the virus are important. However, this disease is difficult to prevent from spreading due to the following characteristics.

Since CyHV-2-infected fish look healthy for multiple days after initial infection (Figure 1) (e.g. 7-10 days depending on conditions), it is difficult to identify and therefore isolate and treat diseased fish. In addition, a behavioural change (lethargy) is observed only a few days before death. These characteristics of CyHV-2 infection may be the

Fig.1 Infected and dead fish in infection experiment by immersion of CyHV-2

This characteristic of CyHV-2 infection may be the reason that goldfish farmers have difficulty in detecting infected fish on-farm.

Table 1. Results of PCR detection of CyHV-2 DNA and of virus isolation from the imported golfish.

Import source	Clinical status	CyHV-2 DNA	Virus Isolation
Singapore	Apparantly healthy	Positive	Positive
China	Apparantly healthy	Negative	Negative
Israel	Apparantly healthy	Positive	Negative
Singapore	Apparantly healthy	Negative	Negative
Hong Kong	Apparantly healthy	Negative	Negative
Singapore	Apparantly healthy	Positive	Negative
Singapore	Apparantly healthy	Negative	Negative
Israel	Diseased	Positive	Negative

reason why the infection might spread in markets, wholesalers and retailers (Ito et al., 2013).

It is suggested that CyHV-2 is spread through dealers including the global trade of goldfish (Table 1) (Ito et al., 2017), and by movements of infected wild fish.

Since CyHV-2 has caused death in goldfish, and wild and cultured Prussian carp, it is difficult to make general prevention measures.

In order to overcome these difficulties, we think that it is important to develop a quick, simple and highly sensitive diagnostic method for detection of diseased fish as quickly as possible, and to provide goldfish lovers/hobbyists with more general information on the characteristics and symptoms of this disease to enable greater awareness of this disease.

Discussion

Sven Bergmann asked, if the virus can be passaged in cell cultures. Takafumi Ito replied, that GFF (Goldfish Fin) cells from goldfish were used, in which the virus was sub-cultivated more than 7 passages. The virus supernatant after primary virus isolation was used for experimentally induced infections and vaccination trials with goldfish.

Perhabdoviruses, a threat for percid farming

L. Bigarré, F. Pozet, Y. Ledoré, P. Fontaine, and F. Lieffrig

Since their first discovery in 1980 and the development of percid farming for the last two decades, viruses from the family Rhabdoviridae have been responsible of high losses in percid farms and experimental facilities in Europe (Belgium, France, Ireland, Norway, Switzerland, etc.) (Figure 2)(Dannevig et al., 2001; Dorson et al., 1984; Nougayrède et al., 1992; Rodger and Girons, 2008; Ruane et al., 2014; Wahli et al., 2015). To date, reports described mostly losses on perch, but several outbreaks have impacted pike-perch production in 2016. The genetic diversity of these viruses (genus perhabdovirus) is high. At least two species are described: perch rhabdovirus (PRhV) and sea trout rhabdovirus (STRV), each species regrouping a number of known variants and probably a larger number yet to be discovered (Talbi et al., 2011). These viruses may affect non-percid fish species which are therefore potential reservoirs in the wild. The transmissions of these viruses are horizontal (fish to fish) and vertical (genitor to fry). Consequently, the control of the disease is based on disinfection of the eggs and prevention of viral introduction in farms. However, both strategies are insufficiently used in Europe. Diagnostics tools are still poorly developed and rely on complementary methods in specialised laboratories: in vitro culture of the virus and PCR. These perhabdoviruses have been considerably neglected these last years, although they are a main infectious threat that should be actively controlled in the future if percid farming is to be developed.

Discussion

Torsten Boutrup asked, if it is possible to access samples from the genitors in the Czech Republic. Laurent Bigarré replied, this is not possible. Anne Nichols from Ghent University asked, how long fish should be kept in quarantine. Laurent answered, two to three days for larvae at the right temperature. If there are no symptoms and no mortality, fish may be transferred. Anne asked then about the quarantine period

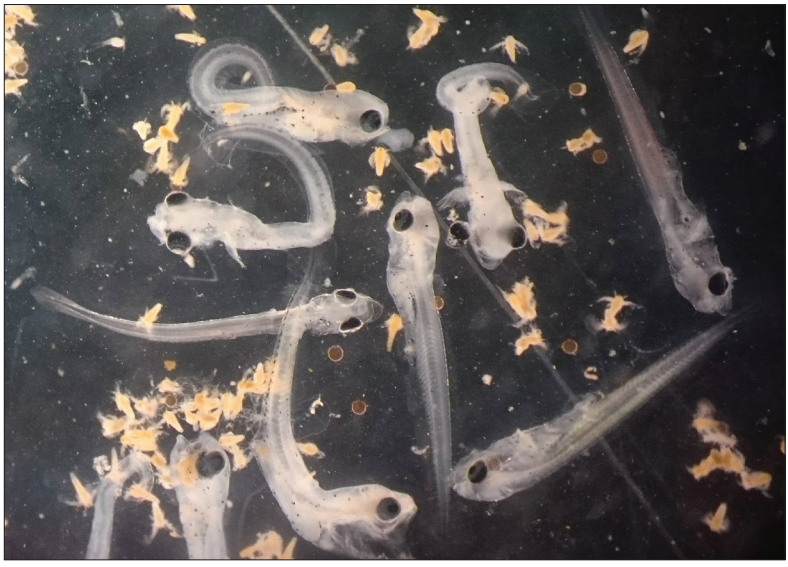

Figure 2. Mortality of pike perch larvae caused by disease through a perhabdovirus (picture Y. Ledoré)

for larger fish. Laurent replied that it is rare to observe symptoms, but it might be needed to quarantine for a longer time (several weeks). Tom Murphy from Dublin asked, how to secure genitors coming from the wild. Laurent replied, that from these fish the virus might emerge. There is currently a need for a diagnostic tool. Tomas Vesély from Czech Republic asked about sensitive cell lines and temperature. Laurent replied, EPC and BF-2, and depending on the laboratory, incubate these at 14 or 21°C.

Piscine orthoreoviruses in farmed rainbow trout: pathogenesis under experimental conditions and diagnostics

Niccolò Vendramin, Helena Hauge, Anne Berit Olsen, Torunn Taskdal, Øystein Wessel, Anna Luiza Farias Alencar, Maria K Dahle, and Niels Jørgen Olesen

A new disease in farmed rainbow trout *(Onchorhyncus mykiss)* was described in Norway in 2013. Farmed rainbow trout suffered unexplained increased mortality, the diagnostic process ruled out known pathogens and described pathology resembling heart and skeletal muscle inflammation (HSMI) in Atlantic salmon *(Salmo salar)*. HSMI is caused by Piscine orthoreovirus (PRV).

Further investigation led to detection of a sequence with high similarity to PRV in diseased rainbow trout. This finding called for a targeted effort to assess the risk the new PRV-variant, recently named PRV-3, poses on farmed rainbow trout by studying transmissibility and disease pathogenesis, and provide information for conducting diagnostics. A cohabitation trial performed in rainbow trout confirmed viral replication in this host, in that the virus levels peaked in blood and heart of cohabitants after 6 weeks post challenge. Heart inflammation was diagnosed in all examined rainbow trout cohabitants 8 weeks after challenge.

In conclusion the novel PRV infects and causes heart pathology in rainbow trout. The infection under experimental conditions develops as acute viral infection, characterised by a viral peak followed by clearance.

Information on the diagnostic tools available and knowledge on viral epidemiology will be provided during the presentation.

Discussion

Mikolaj Adamek informed,that they have PRV-3 and PRV-1 in Germany from an outbreak in rainbow trout. Niccoló requested him to send the isolates to the EURL for Fish Diseases. Mikolaj asked, if you see also mortality in small fish. Niccoló replied, he did not know, but that this disease is normally stress related, like with transport, sea transfer, etc.. Sven Bergmann asked, what is the relation of PRV to eel, carp, koi, and grass carp reovirus that would grow in cell culture. Niccoló replied, that the viral species mentioned by Sven belong to the group of aquareoviruses, and not to orthoreoviruses. Currently it is not possible to cultivate piscineorthorevirus in cell culture. Sven inquired about the detection of PRV outside Europe. Niccoló replied that HSMI is reported in Chile. In Canada, PRV has been detected but HSMI has been reported only once in British Columbia.

Carp edema virus disease (CEVD) in Europe

Olga Haenen

An outline of the current status of CEV detections in carp and koi *(Cyprinus carpio)* in Europe will be presented with some research results, based on the alert paper in *Dis. Aquat. Org*. 2017, written by experts from 15 institutions in 11 European countries and USA: *Way K., O. Haenen, D. Stone, M. Adamek, S.M. Bergmann, L. Bigarré, N. Diserens, M. El-Matbouli, M.C. Gjessing, V. Jung-Schroers, E. Leguay, M. Matras, N.J. Olesen, V. Panzarin, V. Piačková, A. Toffan, N. Vendramin, T. Veselý, T. Waltzek*: The emergence of carp edema virus (CEV) and its significance to European common carp and koi, *Cyprinus carpio*.

Carp edema virus (CEV) disease, also known as koi sleepy disease, is caused by a poxvirus associated with outbreaks of clinical disease in koi and common carp. It was originally characterised in Japan in the 1970's. International trade in koi has led to the spread of CEV. In 1996, CEVD was reported in the USA. In 2009, the disease was first recognised in Europe. Since its first detection in Europe, detection and diagnostic methods improved, and more EU member states reported CEV associated with disease outbreaks. So far, in Europe, Austria, Czech Republic, France, Germany, Italy, Poland, Switzerland, The Netherlands, and the UK have detected CEV.

The genome of the virus has been partly sequenced, and suggested the existence of distinct geographical populations of CEV infecting both koi and common carp. New qPCR primers successfully detected CEV DNA in formalin-fixed, paraffin-embedded archive material from investigations of unexplained carp mortalities of over 15 years ago.

Disease management and control methods, and biosecurity, good health management and disease surveillance, applied to koi herpesvirus disease, can be equally applied to CEVD. There is little chance CEVD would be considered for notifiable disease listing. However, governments might instigate disease control meas-

ures. Further research is needed to develop susceptible cells to isolate CEV, and to study the disease pathogenesis and epidemiology to estimate the likely impact of CEVD on European koi and common carp aquaculture and on wild carp stocks.

There is an active CEV network which organised two workshops (EURL, 2015a, 2015b). Please contact Olga Haenen to be added to the mailing list.

Discussion

Mikolaj Adamek invited the audience to attend his presentation on CEV in gill disease at this conference. Sven Bergmann worked on the diagnostics, based on the old Oyamatsu PCR: In India and China there are varieties and differences in strains found. It would be good to investigate different strains circulating in warm water in Europe. Bartolomeo Gorgoglione inquired about the status of the Full genome sequencing of CEV. Mikolaj Adamek replied, they are nearly ready. Maria Dahle told, her laboratory at Norway works on SGPDV (Salmon Gill Pox Disease Virus) and that they are struggling finding cell cultures and similar challenges. She invited the audience to cooperate. They have finished transcriptional analysis, and have interesting findings. This was warmly welcomed by the audience.

The potential role of fish endogenous retroviruses in disease emergence

Jean-Christophe Avarre

This presentation raised an interesting question that is often overlooked in the field of viral (re) emergence: the potential role of endogenous retroviruses in fish diseases. While several exogenous retroviruses have been unambiguously identified as aetiological agents of some fish diseases (Quackenbush, 2016), no clear association between endogenous retroviruses and diseases has been established in fish so far, perhaps with the exceptions of the salmon leukemia virus, which may have an endogenous origin (Eaton et al, 1994), and the zebrafish endogenous retrovirus, which may be involved in the zebrafish T-cell leukemia (Frazer et al, 2012) (Table 2). The advent of next-generation sequencing has contributed to the accumulation of large fish genomic datasets. Scrutiny of these latter has started to uncover new types of endogenous retroviruses, some of which with a potential for retained infectivity or re-emergence, as is the case in other animal groups (Naville and Volff, 2016). These new findings were presented and discussed.

Discussion

Sven Bergmann asked if there is a possibility that fish retroviruses become zoonotic in the future. Jean-Christophe Avarre replied that nothing was known about that so far, and it is just starting to be studied now. In murine animals, endogenous retroviruses may be transmitted between different species (Stocking and Kozak, 2008). Torsten Boutrup added that there could be a risk for pathogens in warm waters, while it was unlikely in cold waters. He added that walleye sarcoma virus was temperature dependent.

General discussion

Which is the most important neglected viral disease in freshwater fish farming?

Jason Mewett from CEFAS answered, potentially this would be CEV. It is a real problem, also related to carp angling in the carp sport fisheries branch. Sven Bergmann adds that losses in eels

Table 2. Retroviruses described in fish (according to Lepa and Siwicki, 2012).

Virus name	Abbreviation	Original host species
Walleye dermal sarcoma virus	WDSV	*Sander vitreus*
Walleye epidermal hyperplasia virus type 1	WEHV 1	*Sander vitreus*
Walleye epidermal hyperplasia virus type 2	WEHV 2	*Sander vitreus*
Perch epidermal hyperplasia virus type 1	PEHV 1	*Perca flavescens*
Perch epidermal hyperplasia virus type 2	PEHV 2	*Perca flavescens*
Salmon swimbladder sarcoma virus	SSSV	*Salmo salar*
Salmon leukemia virus	SLV	*Oncorhynchus tshawytscha*
Snakehead retrovirus	SnRV	*Ophicephalus striatus*
Zebrafish endogenous retrovirus	ZFERV	*Danio rerio*

due to rhabdovirus, reovirus and picornavirus are also important, and need increased attention for the eel sector.

Are diagnostic methods up to date, which ones to use?

There are various PCR tests possible to use. The Way et al. paper (2017) gives information on the possible and best diagnostic methods at various laboratories. The development of susceptible cell lines is now crucial to have tools for further development of diagnostic tests, such as serological tests, like ELISA, and for replicating the virus for pathogenicity and pathogenesis studies.

Are prevention measures in place, and which specific vaccines are needed?

For development of a vaccine, for CEV we first need susceptible fish cell lines. Sven Bergmann added that mononuclear cells are needed. Olga asked about the need for vaccines regarding perch viruses. Laurent answered that there is a need for diagnostic tests, and to improve and increase egg disinfection. Eggs of perch are sticky and are therefore difficult to disinfect, although egg disinfection is easy for pike perch eggs. Torsten Boutrup advised, we need to have a quality check to secure disinfection protocols. Uwe Fischer from FLI stated, for rhabdoviruses, DNA vaccination is a good option with other rhabdoviruses than from perhabdoviruses. Recombinant vaccines could also be an approach for CEV. Eva Lewisch from Vienna underlined the importance that, for prevention, we need to validate protocols and know how long the virus will persist in the environment.

What recommendations can be made for effective diagnosis and prevention of neglected viral disease problems in freshwater fish farming?

It is recommended to form a group of experts to sequence various pathogens and set up an international platform. Jean-Christophe Avarre agrees on such a platform, and emphasised that with the advent of 3rd and 4th generations of sequencing, it is important to have a pipeline (see Bayliss et al. (2017) for illustration). Richard Paley from CEFAS will seek UK national funding. Olga Haenen hopes that the current publication provides necessary basic information on these emerging and neglected viruses to raise appropriate funding for future research initiatives.

Overall conclusions

It was concluded that this workshop was very useful to address neglected viral diseases of freshwater fish farming, which should be strongly considered for their ability to emerge in the near future.

Acknowledgements

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