Report about fish viral diseases

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Report about fish viral diseases

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About diagnostic laboratories for fish viral diseases

A total of 27 laboratories in 9 different countries state that they perform virological studies. However the scrutiny of the answers determines that only 13 laboratories from 6 countries (Croatia, France, Greece, Italy, Romania and Spain) regularly perform virological diagnosis. A number of countries (Cyprus, Egypt, Morocco, Portugal and Turkey) also report on viruses but the laboratory involved in the analysis is not identified. Many analyses for virus are delegated to other country or different laboratories.

Most reports about virus comes from finfish species produced intensively in Mediterranean countries, both from marine species (seabass, seabream and turbot that represent about 22% of fish production) and from freshwater (trout that represent 29% of the total fish production). Very scarce information was obtained on the viral diseases occurring in the main fish species produced in extensive and semi-intensive systems, i.e. tilapia, carp and mullet, that represent about 50% of the fish production in the region.

Main reported diseases

A total of 9 viruses – lymphocystis, nodavirus, infectious pancreatic necrosis (IPN), infectious hematopoietic necrosis (IHN), viral hemorrhagic septicemia (VHS), spring viraemia of carp (SVC), catfish iridovirus, catfish herpervirus and eel herpervirus – are reported to be present in the area for the years 1998, 1999 and 2000, although only 6 represent some threat.

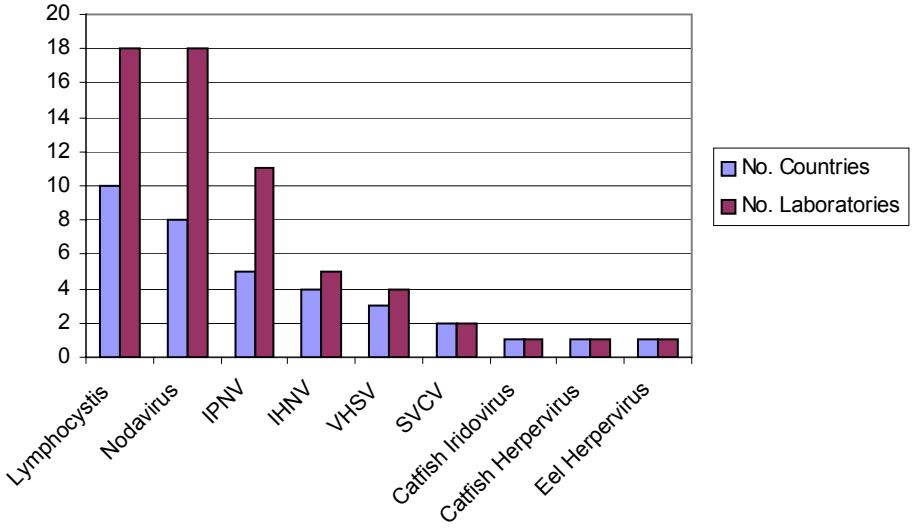

Fig. 1. Reported virus in the Mediterranean region.

The lymphocystis iridovirus in seabream is the virus most frequently reported (10 countries and 18 laboratories), followed by the nodavirus from seabass (eight countries and 18 laboratories). IPNV is

reported in 5 countries, IHNV in 4 and VHSV in 3. SVC is reported twice, only in France and Romania. Finally, the catfish herpesvirus and the catfish iridovirus is stated by only 1 laboratory in Italy.

It seems to be an overestimation in the diagnosis of lymphocystis and nodavirus with respect to other viral diseases due to the easy diagnosis of these two virus, which is based on clinical signs, macroscopical examination and histophatology.

It is recalled that this survey does not form part of a disease/pathogen reporting system, and that validated information for country sanitary status, especially for notifiable diseases, should be obtained either from the relevant national authorities or the OIE. Three rhabdovirus (IHNV, VHSV and SVCV) from the 9 viruses reported in the survey are classified within the OIE list of notifiable diseases. Other OIE significant diseases also stated in the survey are the viral encephalopathy and retinopathy (nodavirus) and the IPN.

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Lymphocystis disease (LD)

The LD of fishes is a well-known viral infection associated with hypertrophy of connective tissue cells presented as nodular skin lesions. LD has been described in many species of cultured and wild marine fish species such as seriola, flounder, gilthead seabream, red seabream, dab, sole and plaice, with a special incidence in gilthead seabream cultured in European Mediterranean countries, and in wild pleuronectid fish recovered in the North Sea and Baltic Sea in European waters.

Taxonomically, lymphocystis virus (LV) belongs to the genus *Lymphocystivirus* within the *Iridoviridae* family. The LV is an icosahedral particle varying in size (from 215-240 nm across the apices) dependent on the fish host origin (Smail and Munro, 2001), and contains double stranded DNA. At present, the Red seabream iridovirus, redfin perch and the white sturgeon iridovirus belongs to a distinct genus (*Ranavirus*).

The LV induces chronic or slowly developing disease, often recurrent, with the highest incidence during the summer months. Although the disease causes very low mortalities, the external clinical signs of affected fish make them unmarketable. In addition, fish affected by LD are more susceptible to other bacterial, viral or parasitic infections.

The best way to control the disease in the farms is to isolate the affected fish as early as possible to prevent cross-contamination and allow the lesions to heal.

Current status based on received answers

The disease was reported by 18 laboratories from 10 countries (Croatia, Cyprus, Spain, Greece, Israel, Italy, Malta, Morocco, Portugal and Turkey) in juvenile seabream (from 5 to 50 g) in both routine monitoring and outbreaks with low mortalities. Although being the most reported viral fish disease in the region, it is not consider to be a major concern.

The diagnosis is based on clinical signs and macroscopical aspect. Recently, cultured sole has been described as new species susceptible to lymphocystis.

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Nodavirosis (viral encephalopathy and retinopathy; VER)

The VER disease, also called viral nervous necrosis (VNN), is caused by the group of piscine nodaviruses (genus *Betanodavirus*) and produces important economic losses in the larval culture of a great number of marine fish species (more than 20) such as seabass, striped jack, halibut and grouper. Sporadic cases have also been reported in turbot, sole, cod and salmon. The disease, as the name indicates, is characterised by a cell vacuolisation and neuronal degeneration in the central nervous system and the retina. Therefore, affected fish show a loss of equilibrium, failure of muscular control and visual dysfunction.

The nodavirus is the smallest single-stranded RNA virus that affects fish (25-34 nm in diameter). It is icosahedral, with a single coat protein and a bi-segmented genome. Although this disease was described for the first time in 1988, until 1996 it was not possible to isolate the etiological viral agent by means of the development of a cell line named SSN-1 from *Chana striatus* (striped snake-head).

The disease causes percentages of mortality ranging from 50 to 100% in the larval stages but these values decrease with fish age. The fish susceptibility to the nodavirus is also dependent on temperature and strain. In fact, genetic studies demonstrated that at least two distinct genogroups exist, each one with a different replication temperature (around 25°C or below 15°C).

Although the nodavirus can be transmitted horizontally by contact between diseased and healthy fish, the main transmission route is vertical, the broodstock being the reservoir of this virus, which is transmitted to the larvae through the fertilised eggs.

The presumptive diagnosis of VER can be made on the basis of the light microscopic appearance of the brain, spinal cord and/or retina. However, individual fish with the presence of only a few vacuoles in the neuropil pose a difficult diagnostic problem. Therefore, the confirmative diagnosis of the disease must be based on the isolation of the virus in the fish cell-line SSN-1 in conjunction with the direct detection of the virus in fish tissues by immunological methods.

The best alternative for the diagnosis of the agent is the utilisation of the reverse-transcriptase PCR (RT-PCR) amplification of a fragment from the coat protein gene, a procedure that is recommended in the diagnostic manual of the OIE.

Attempts to control the disease by vaccination strategies are currently being conducted. The VER is considered as a "significant disease" by the OIE but it is not included in the EU legislation. One of the two OIE reference laboratories for this diseases is in the Mediterranean region, that of Dr. G. Bovo (IZSV, Italy, Record No. 30).

Current status based on received answers

Eighteen laboratories from 9 different countries (Croatia, Cyprus, Spain, France, Greece, Israel, Italy, Malta and Turkey) report the virus in both routine monitoring and mortality cases. The main species affected is seabass, but some cases occur in shi-drum (*Umbrina cirrosa*) and mullets as well as in wild and cultured grouper (*Epinephelus marginatus*). Some laboratories report severe mortalities in hatcheries, in larval and juvenil stages of seabass.

The techniques more frequently used to diagnose the disease are clinical signs, histopatology, cell culture and PCR.

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Infectious pancreatic necrosis (IPN)

IPN is an acute contagious systemic disease. The causative agent is the IPN virus (IPNV), a bi-segmented double-stranded, icosahedral, naked RNA virus which belongs to the genus *Aquabirnavirus* within the family *Birnaviridae*. Although this group was classically known for their high incidence in salmonids cultured in fresh water (where the viruses can cause 100% mortality in 1-4 month old fish), at present it is accepted that the birnavirus have a wide distribution in fresh and marine waters affecting fishes, molluscs and crustacea. Although 10 serotypes have been described in IPNV, most of the disease problems are caused by three of them: Sp, Ab (classic European serotypes) and VR-299 (West Buxton) (classic American serotype).

Regarding the incidence of IPNV in the marine cultures, the virus has been associated with mortalities in Atlantic salmon in Norway and Scotland, especially during the first months after their transfer to seawater. At present, IPNV continues to be a problem for the post-smolt salmon cultured in seawater cages. Moreover, sporadic cases of infections by birnavirus have been described in strictly marine fish such as seabass in France, turbot in Norway, France and Spain, cod in Denmark, and sole in Spain. However, IPNV is frequently isolated from halibut in Norway and Scotland often associated with mortalities occurring during weaning. It is noteworthy that in these marine fishes, unlike in salmonids, the pancreatic tissue is not altered.

In relation to the possible origin of the birnavirus in the marine cultures, it has been suggested that wild fish or invertebrates may be important vectors. Conversely, pisciculture plays a role in the transmission of this viral group in the marine environment. In fact, birnavirus has been isolated from the hepatopancreas of scallops and mussels collected around the aquaculture facilities, from wild marine fish formerly used to prepare moist pellets in those farms, as well as from rotifers and *Artemia* used as live food in the larval stages of marine fish species.

On the other hand, IPNV presents a long survival in the aquatic environment, which indicates that the virus possesses capacity of transmission from carrier or clinically ill fish in rearing facilities to susceptible hosts far away from the source of infection. In addition, vertical transmission of the virus via the reproductive products has been demonstrated.

The preliminary diagnosis of IPN can be conducted by the isolation of the virus in susceptible cell lines like CHSE-214 (from chinook salmon embryo). However, a confirmative identification of the viral isolates must always be performed by immunological or RT-PCR procedures. In addition, the RT-PCR can be applied directly to recent infected cell lines and/or fish tissues. Although many primer pairs for RT-PCR can be found in the literature, most of them directed to segment A of the RNA genome, only the "Jav" set which amplify a region of 607 bp in this segment allows the detection of strains from any serotype. In addition, the RT-PCR combined with the restriction fragment length polymorphism analysis (RFLP) using the enzymes Pvu II, and further if necessary, Eco RI, Bst EII or Mbo I, proved to be a powerful epidemiological tool for typing the IPNV strains.

Control methods are currently based on the implementation of control programmes, through the avoidance of the introduction of fertilised eggs. To prevent mortalities by IPNV in Atlantic salmon in European Nordic countries, a recombinant vaccine has been developed which is administered together with bacterins against vibriosis and forunculosis as polivalent vaccine.

The IPN is considered as a "significant disease" by the OIE, and it is included in list III of the EU legislation.

Current status based on answers received

In a total of 11 laboratories from 5 countries (Spain, France, Italy, Portugal and Turkey), the virus is detected in routine monitoring and mortality cases. The main species affected are the salmonids (rainbow and brown trout). Some cases were reported in monitoring of salmon, eel, turbot and seabass. Although not by the survey, the virus has also been recently diagnosed in Greece.

The techniques to diagnose the presence of the virus are cell culture, together with seroneutralisation and also PCR.

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Infectious hematopoietic necrosis (IHN)

Although the principal clinical and economic consequences of IHN occur in farms rearing rainbow trout in freshwater, both Pacific and Atlantic salmon cultured in freshwater or seawater may be severely affected. Large mortalities have also been recorded among some wild stocks of Pacific salmon. Therefore, a reservoir for the causative virus is suspected among marine fish residing in coastal areas of the eastern Pacific Ocean.

Historically, the geographical range of IHN was limited to the western parts of North America, but the disease has spread to continental Europe and the Far East via the importation of infected fish and eggs.

The etiological agent responsible for this disease is an enveloped single stranded RNA virus belonging, as in the case of VHSV, to the genus *Novirhabdovirus* within the *Rhabdoviridae* family.

On the basis of antigenic studies conducted with polyclonal antisera, IHNV isolates form a single serogroup. However, monoclonal antibodies to the glycoprotein and nucleoprotein have revealed a number of antigenic variants among the strains of the rhabdovirus. In addition, variations in virulence of IHNV strains have been recorded during both natural cases of disease and experimental infections. Analyses of the genetic diversity of IHNV have revealed the existence of a number of genogroups.

The IHNV in susceptible fish causes viraemia with subsequent destruction of internal organs, impairment of osmotic balance, edema and haemorrhage. These clinical signs are a consequence of the high virus multiplication in endothelial cells of blood capillaries, haematopoietic tissues and nephron cells.

The transmission of IHNV between fish is primarily horizontal because the virus is shed via faeces, urine, sexual fluids and external mucus from both clinically infected fish and covert carriers. Although the horizontal transmission is typically by direct exposure, invertebrate vectors have been proposed to play a role in some cases. Vertical or egg-associated transmissions have also been demonstrated, being the only mechanism accounting for the appearance of IHN in new geographical locations. This egg-associated transmission is significantly reduced by the common practice of surface disinfection of eggs with iodophors.

The age of the fish appears to be extremely important to the susceptibility to the IHN: the older the fish, the more resistant to disease. In addition, the most prominent environmental factor affecting IHN is water temperature, clinical disease occurring between 8 and 15°C.

The screening procedure for IHNV is based on virus isolation in susceptible cell lines [EPC (epithelioma papulossum of carp) and BF-2 (blue gill fin)]. Confirmative identification may be achieved by use of immunological or molecular (DNA probe or RT-PCR) methods. The primer sets recommended for the successful detection of IHNV in the RT-PCR procedure are directed to the nucleoprotein (N) gene sequences conserved among all known isolates of this virus but not present in the N gen of the related rhabdovirus, VHSV.

At present, as with the other marine fish rhabdovirus VHSV, vaccination to prevent IHN is at an experimental stage; however, several new vaccine preparations based on the recombinant DNA technology have shown promising results in both laboratory and field trials.

The IHN is considered as a "notifiable disease" by the OIE (OIE, 2000) and it is also included in list II of the EU legislation.

Current status based on received answers

The presence of this rhabdovirus or the occurrence of the disease was reported in 5 laboratories from 4 countries (Spain, France, Italy and Turkey). The species involved are cultured and wild salmonids, mainly rainbow and brown trout. In the case of Spain is the stated only the presence of the virus in wild salmon.

The techniques used are cell culture, seroneutralisation and PCR.

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Viral haemorrhagic septicaemia (VHS)

This viral septicaemia was classically considered as the most serious viral disease of farm salmonids cultured in freshwater in northern European countries. However, since 1980, epizootics of VHS occurred also in rainbow trout cultured in seawater as well as in turbot in Germany, Scotland and Ireland.

Although until 1989 the virus was considered endemic of Europe, from this year different strains of this virus are being repeatedly isolated in North America from Pacific salmon returning from the ocean migration to spawn in the rivers. Later, the VHSV has also been recovered in both Pacific and Atlantic Oceans from wild and cultured marine fish such as cod, pacific herring, sprat, rockling, pollack, flounder and halibut which seems to indicate that wild marine fish are the main reservoir of this virus.

The pathogen responsible for the disease is an enveloped single-stranded RNA virus belonging to the recently approved genus *Novirhabdovirus* within the *Rhabdoviridae* family.

The genetic comparison of strains of VHSV from Europe and North America indicated that they constitute two separate clonal lineages, which demonstrated that the American isolates do not have a European origin. Moreover, within the European marine environment distinct genogroups of VHSV can be identified. The marine isolates are not pathogenic for the salmonids and viceversa, the salmonid isolates are not pathogenic for marine fishes.

The age of the fish and water temperature influence susceptibility to VHS. In general the susceptibility to VHS decreases with age, although overt infections are seen in all sizes of fish. Disease generally occurs at temperatures between 4 and 14°C with the highest mortalities resulting between 1-5°C. The clinical signs of affected fish are the widespread haemorrhaging seen internally over the liver, adipose tissue and especially, within the muscle. Externally, the fish may exhibit skin haemorrhages, exophthalmia in one or both eyes, and there may be haemorrhaging around the eye orbit.

The screening procedure for VHS is based mainly on virus isolation in cell cultures such as EPC and BF-2 fish lines. Confirmatory testing is by immunological virus identification (i.e. immunofluorescence, ELISA) or by RT-PCR based technology using different primer sets directed to regions between G and NV genes or within the NV gene. The PCR is the most suitable procedure to detect the virus in fish tissue with overt disease.

Horizontal and vertical transmissions of VHSV have been demonstrated. The virus is shed in the faeces, urine and sexual products. Once VHSV is established in a farm and, therefore, in the water catchment system, the disease becomes enzootic because of the virus carrier fish.

In recent years, there was an increased effort by several laboratories to produce an efficient subunit or single virus protein vaccine or DNA vaccine using the available recombinant DNA technology. However, at present none of these vaccines are licensed and commercialised to be used in the field.

The VHS is considered as a "notifiable disease" by the OIE and it is also included in list II of the EU legislation.

Current status based on received answers

The presence of this rabdovirus was reported in 4 laboratories belonging to 3 countries (Spain, Italy and France). The disease is stated in France and Italy, whereas in the case of Spain is just the presence of the virus in wild asymtomatic fish what is stated. The species affected are cultured and wild salmonids, pike, black bass and wild Iberian nase.

The techniques used were cell culture, seroneutralisation and RT-PCR.

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Spring viraemia of carp (SVC)

Spring viraemia of carp is an important disease affecting cyprinids and is reported to be present in several European countries. Although the common carp (*Cyprinus carpio*) is the main host of SVC virus, natural outbreaks of SVC were also found in other species of freshwater fishes. Recently, the first case of SVC was confirmed during June 2002 in the East coast of the United States. Mortality rates of young carp due to SVC can reach up to 70% during spring outbreaks, but the yearly losses of older fish are usually bellow 39%. Outbreaks of SVC depend on the temperature of water, age and condition of fish, population density and stress factors.

Clinical disease dominates at water temperature between 5 and 10ºC but mortality rates are faster typically in spring, at temperatures between 10 and 17ºC. At higher temperatures, infected carp are able to develop antibodies and the fish become protected against re-infection. External signs of SVC are darkening of the skin, abdominal swelling, exophthalmia and petechial haemorrhages in the skin, gills and eyes. Internally, haemorrhages in the swim bladder, oedematous organs, ascitis and catarrhal enteritis are usually seen.

Transmission of SVCV is generally horizontal. Excretion of virus via faeces and urine from infected fish has been demonstrated. In addition, biological vectors such as leeches, carp louse (*Argulus foliaceus*) as well as fish-eating birds such as herons can play a role in the transmission of the disease.

The etiological agent (SVCV) is presently classified as a tentative member of the genus *Vesiculovirus* of the family *Rhabdoviridae*.

SVC is listed as a notifiable disease by the OIE. Diagnostic procedures should be based in the OIE guidelines. Etiological diagnosis is performed by the demonstration of virus presence in tissue samples using electron microscopy or immunofluorescence (IF) or by the isolation of the viral agent. For the latter, cell lines such as FHM (fathead minnow) or EPC are recommended. The isolated virus can be identified by serum neutralisation test, IF, immunoperoxidase or ELISA.

SVCV is serologically distinct from the other known fish rhabdoviruses, with the exception of pike fry rhabdovirus (PFR) that cross-reacts with SVCV by IF and ELISA. To distinguish between both viruses, a ribonuclease protection assay was developed. Recently a RT-PCR procedure allows the cyprinid rhabdovirus to be divided into four differentiated genogroups, the SVC being included in genogroup I and the PFR in the IV.

In rearing facilities with a controlled environment, elevation of water temperature can prevent or stop SVC outbreaks. Effective and safe immunoprophylaxis has not yet been established. However, carp vaccinated intraperitoneally (ip) or orally in autumn with live virus can develop a resistance to reinfection, which can last several months. A commercial inactivated SVCV preparation gave positive results in ip vaccination of carp in Eastern Europe. However, for fry and fingerlings only bath or oral delivery is possible in the present carp production technology. Although DNA vaccines show great promise for use against VHSV and IHNV, an efficacious DNA vaccine against SVCV has not yet been reported, due at least in part to the difficulty of the live-fish challenge-model in carp.

Current status based on received answers

The SVC rhabdovirus is reported only to occur in France and Romania, in *Cyprinus carpio* and *Hypophthalmichthis molitrix*. No data were reported from the main producer countries of carp in the region (Egypt and secondarily, Israel), the possible reason being that the disease is described in countries/areas with colder waters.

The diagnosis carried out is done by ELISA technique.

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