

Monitoring of **Category 1 health hazards for fish** in 2014: a stable situation

Thibaud Roman (1) (thibaud.roman@agriculture.gouv.fr), Joëlle Cabon (2), Marine Baud (2), Lénaïg Louboutin (2) Laurent Bigarré (2), Thierry Morin (2)

(1) Directorate General for Food, Animal Health Office, Paris, France

(2) ANSES, Ploufragan-Plouzané Laboratory, Viral Diseases in Fish Unit, France

Abstract

The concomitant intensification of single-species breeding in the aquaculture industry and the trade of fish and eggs have complicated fish farm health management in production areas by encouraging the emergence and spread of pathogens such as rhabdoviruses, responsible of Viral haemorrhagic septicemia (VHS) and Infectious haematopoietic necrosis (IHN). Appropriate monitoring was set up starting in the 1990s in order to more effectively assess the health situation. Surveillance applies to salmonid farms (which rank first in French fish production), as well as to pond-based fish farming, due mainly to the presence of pike, a typical species for this biotope and a carrier of VHSV. In 2014, monitoring results confirmed the maintenance of a stable health situation in France with regard to VHS. Two silent outbreaks of IHN were detected and eradicated. Two outbreaks of Koi herpes virus (KHV) were also detected, confirming the contamination of our national carp population by this disease which has been detected regularly for over a decade.

Keywords

Fish, Viral diseases, Category 1 health hazards, VHS, IHN, KHVD, ISA

Résumé

Surveillance des dangers sanitaires de première catégorie pour les poissons : une situation stable pour l'année 2014
La généralisation de l'élevage mono-spécifique intensif dans la filière piscicole et l'intensification des échanges de poissons dans les bassins de production en favorisant l'apparition et la diffusion d'agents pathogènes tels que les rhabdovirus, responsables de la septicémie hémorragique virale (SHV) ou de la nécrose hématopoïétique infectieuse (NHI). Une surveillance appropriée a été mise en place à partir des années 1990 pour tenter de mieux appréhender cette situation sanitaire. Cette surveillance concerne en premier lieu les élevages de salmonidés (qui occupent la première place dans la production piscicole française), mais également la pisciculture d'étang, en raison de la présence d'une espèce typique de ce biotope, sensible au virus de la SHV : le brochet. Les résultats de la surveillance en 2014 confirment le maintien d'une situation sanitaire stable sur le territoire vis-à-vis de la SHV. Deux foyers silencieux de NHI ont été détectés et éradiqués. Deux foyers d'herpès-virose de la carpe (HVC) ont été détectés, confirmant la contamination de notre cheptel national de carpes par cette maladie détectée régulièrement depuis plus d'une décennie.

Mots-clés

Poissons, maladies virales, dangers sanitaires de 1^{ère} catégorie, SHV, NHI, HVC, AIS

In fish, four non-exotic viral diseases, previously classified as notifiable diseases with compulsory control measures (NDDCM), are henceforth defined as Category 1 health hazards, under the terms of Decree 2012-845 of 30 June 2012 (Table 1). Three of these diseases are endemic in France. Among them, viral haemorrhagic septicaemia (VHS) and infectious haematopoietic necrosis (IHN) are currently the most important. The third, Koi herpes virus (KHV), has been detected sporadically in France since 2001, and the number of reports seems to be on the rise since 2011 (Papin *et al.*, 2012). France is officially free of infectious salmon anaemia (ISA).

These regulated diseases have been subject to surveillance since Directive 2006/88/EC came into effect, in response to the health requirements set by European regulations to protect fish farms and to

facilitate trade. Note that the list of diseases (Annex IV, Part 2 of this Directive) was amended by an Implementing Directive (2014/22/EU) during 2014, with the introduction of a distinction between genotypes of the ISA virus. For ISA, this list now targets only the pathogenic strains with a deletion in the highly polymorphic region (HPR) of the viral genome.

Surveillance of these diseases is based on a dual system: mandatory surveillance (outbreak and programmed surveillance) and a voluntary scheme (targeted surveillance through programmes to achieve disease-free status) (see Box).

Table 1. Classification of regulated fish diseases, their pathogens and the health situation in France on 31 December 2014

Disease	Pathogen	Regulations	Health situation on 31/12/2014
Viral haemorrhagic septicaemia (VHS)	Rhabdovirus	Category 1 health hazard (formerly NDDCM)	Present
Infectious haematopoietic necrosis (IHN)			Present
Koi herpes virus disease (KHVD)	Herpesvirus		Present
Infectious salmon anaemia (ISA) HPR-deleted genotype	Orthomyxovirus		Absent

Results of surveillance in 2014

In the framework of outbreak and programmed surveillance, a total of 2,058 analyses (1979 by cell culture and 79 by PCR) were performed by the accredited laboratories and the NRL in 2014 (a rise of 2.1% compared with 2013 and of 17.4% compared with 2012; source NRL).

Surveillance of VHS

Three outbreaks of VHS in rainbow trout were reported in 2014 in the framework of outbreak surveillance, one in the Meuse *département* and the two others in Moselle. The last two outbreaks, which occurred in neighbouring farms, were epidemiologically related, as the two viral strains isolated were genetically identical. The phylogenetic analysis of viral strains (Figure 1) also shows that the two viruses isolated in the Meuse and the Moselle are almost identical and very probably have a common origin. The epidemiological investigation conducted by the DDecPPs of the Meuse and the Moselle as well as by the aquaculture resource person for the region based at the DDecPP of the Meuse, with

assistance from the DDecPP of the Territory of Belfort, revealed that pike are strongly suspected to be at the origin of the contamination in the Meuse. This investigation was not able to identify the precise origin of the original outbreak, however.

Comparisons of the sequences of the gene coding for the viral glycoprotein (Figure 1) show that the same strain is probably at the origin of the three outbreaks: 99.8% similarity between the strain isolated in the Meuse and those from the Moselle outbreaks (as these last two sequences are 100% identical, they are represented by the single taxon TAC\FR-57\2014) was observed. They also confirmed strong similarity (98.4%) with a sequence isolated in 2004 from pike (Br.\Fr-63\2004) and a viral sequence isolated in the Vienne *département* in 2013 (TAC\Fr-86\2013).

Finally, the viral sequences isolated in 2014 are very close to a viral sequence isolated in Germany in 2002 (TAC\DE\2002) (Figure 1).

Surveillance of IHN

Two outbreaks of IHN detected through targeted surveillance were reported in 2014, without any clinical suspicion having been raised in the two farms affected. One outbreak was detected in the Doubs *département* following self-inspection and the other was detected in the Manche *département* following an analysis for the purpose of disease-free certification. Since the hatcheries of these farms benefited from effective isolation measures and the juvenile stages are thus protected from infection, the viral infection was latent and silent in the sub-adult and adult stages in both cases.

Surveillance of KHVD

Two outbreaks of KHVD were reported in 2014, following the observation of abnormal mortality in carp. One outbreak occurred in a pond in the Pas-de-Calais and the other in a private basin of Koi carp in Saône-et-Loire. The epidemiological investigation carried out by the relevant *départements* of the DDecPPs of Pas-de-Calais and Saône-et-Loire, and the aquaculture resource person for the region, based at the DDecPP of Pas-de-Calais, concluded that contamination may have originated with recently-imported Koi (ornamental) carp.

Disease-free certification of fish farms regarding VHS and IHN

Four additional fish farm units or areas (i.e. a total of nine fish farms) were declared free of VHS and IHN in 2014. On 31 December 2014, 408 fish farms were certified free of VHS and IHN out of a total of 621 freshwater aquaculture sites identified in 2008 (Agreste, 2011). To these sites can be added an unknown number of ponds, estimated to be in the tens of thousands.

Costs

With data available this year for all 101 *départements*, the sum of €4,452 was spent in 2014 under the surveillance programme to finance outbreak-related visits (veterinary fees and analysis costs) and €13,067 for visits to evaluate the disease-free status of fish farms, including €12,475 in analysis costs. The cost of these health control measures was €38,457 (compensation for slaughter or disinfection). All these operations cost the state a total of €68,715 in 2014, excluding rendering costs.

Discussion

The number of outbreaks of VHS in 2014 returned to a level comparable to previous years (Figure 2), after the peak of 2013 when an infected farm had transmitted the virus to many other sites, which each became secondary outbreaks. (Roman *et al.*, 2014). The epidemiological investigation following the outbreak in the Meuse in 2014 incriminated pike as a vector, a species found in association with viral sequences isolated in 2014 that segregate together in the phylogenetic analysis (Figure 1). The role of pike as a reservoir of the VHS virus, discussed recently (Roman *et al.*, 2013), is here again a topical issue. It is therefore recommended that salmon farming be rigorously separated from the pond-based fish-farming sector in order to reduce the risk of contamination. The strong similarity between the viral sequences analysed in strains in 2014 and a sequence from a viral strain isolated in Germany suggest epidemiological links between the farms of the two countries.

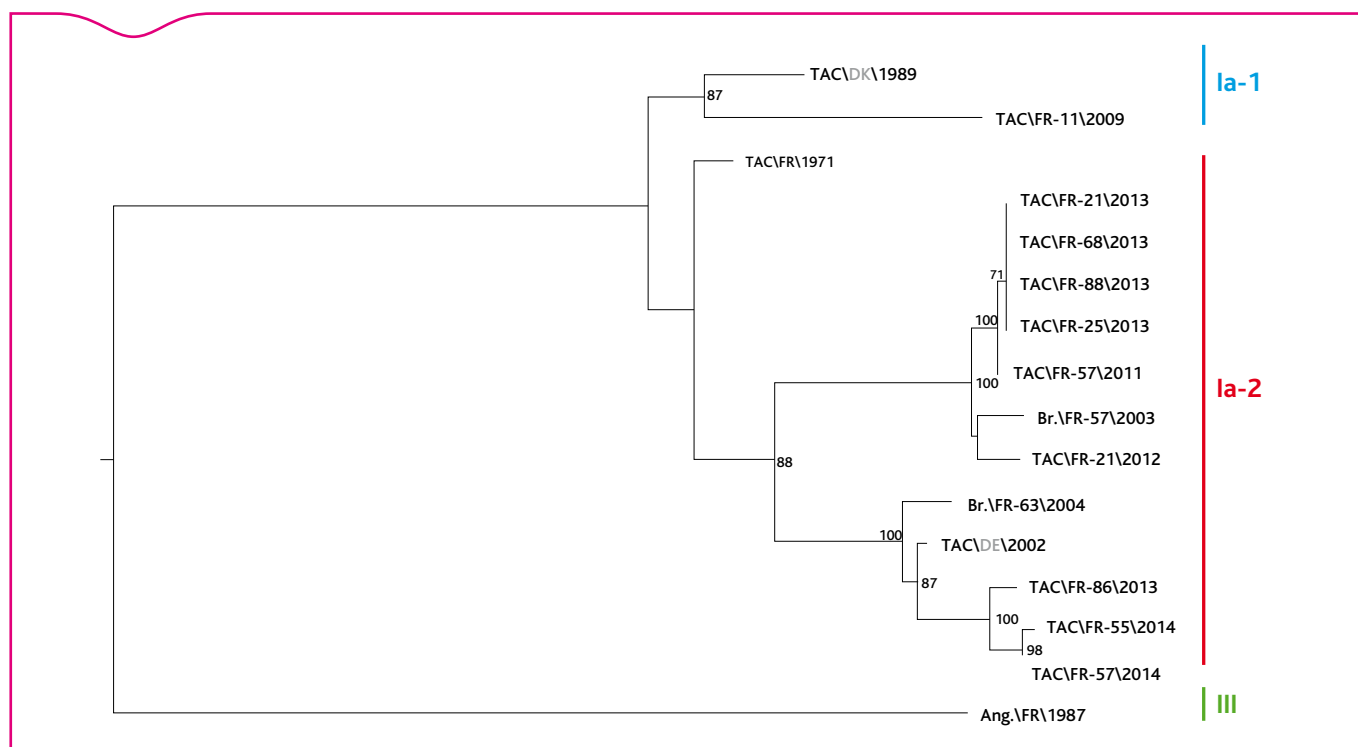


Figure 1. Groups of sequence similarity for the envelope glycoprotein (1524 nt) of the VHS virus.

Each isolate is identified by the following code: Host species (in French)/country-*département*/year. Br: Pike; TAC: rainbow trout; Ang: eel. Phylogenetic analysis created with SeeView in PhyML with 100 bootstrap replicates, using the GTR model. Only bootstrap scores greater than 70 are shown. The classification into genogroups (I, II or III) is based on Kahns *et al.* (2012).

The suspected under-declaration of IHN suggested in 2014 (Roman *et al.*, 2014) is confirmed (Figure 2). This disease can go unnoticed during clinical examination if the more susceptible juvenile stages are protected by careful containment. The factors that tend to foster under-reporting of outbreaks include the low number of veterinarians specialised in aquaculture, the lack of awareness of some fish farmers and fish keepers, professionals and amateurs alike, and the lack of compensation for the value of fish lost to disease when the fish farmer was not involved in a programme to achieve fish disease-free status.

The generalisation of animal health certification and the multiplication of inspections for disease-free certification should progressively improve the detection of regulated diseases, in particular IHN, as a consequence of the resulting surveillance schemes.

KHV was sporadically detected in France in 2001 and 2002, but recurrent outbreaks have been reported since 2008, suggesting that either the virus has become established in France or infected fish are repeatedly being introduced. This disease should henceforth be monitored more closely.

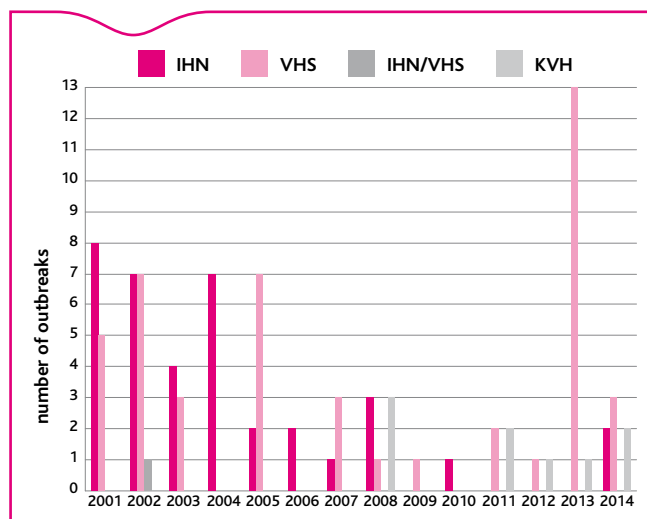


Figure 2. Changes in the number of outbreaks of regulated fish diseases reported since 2001

Box. Surveillance and health control measures for regulated fish diseases

Objectives of the surveillance programme

- To early detect any outbreak of a regulated disease.
- To confirm France's official disease-free status for infectious salmon anaemia (ISA).
- To grant "disease-free" status to aquaculture areas and farms (fish farms, pond-based aquaculture) in order to protect farms (from VHS, IHN and KHV) and facilitate trade.

Monitored population

Farmed and ornamental fish.

Surveillance procedures

Outbreak surveillance

Reporting of any suspected or confirmed cases to the DDecPP (or the DDAAF in French overseas *départements*), in the event of abnormal mortality or observation of clinical signs suggestive of a regulated disease. If any suspicion is declared, samples are taken for first-line analysis by one of the seven accredited laboratories and, if necessary, results are confirmed by the NRL at the ANSES Ploufragan-Plouzané laboratory (by identification of the virus using cellular and/or molecular methods).

Programmed surveillance

Since 2011, outbreak surveillance has been supplemented by the implementation of an animal health certification programme for aquaculture farms. This certification, which is mandatory, is issued by the relevant local authority (DDecPP or DDAAF). It requires that the person responsible for the aquaculture farm carry out a risk analysis and draw up a corresponding health surveillance scheme that includes regulated diseases. Clinical inspections by an accredited veterinarian and audits by the relevant authority are scheduled at a frequency depending on the level of risk determined for the fish farm (from one per year to every 4 years in the framework of a procedure to maintain disease-free status for zones or compartments where the risk level is high or low, respectively). Samples are analysed in the event of suspicion.

Targeted surveillance: (voluntary) disease-free certification programmes of fish-farming zones and compartments

Professionals may set up voluntary programmes for acquiring "disease-free" status focused on a single farm or a larger area including several farms and natural aquatic areas, as stipulated in EU regulations. The farmer may choose either a short programme with extensive sampling (two clinical inspections and two samples of 150 individuals each, once a year for two years), or a longer programme with less intense sampling

(two clinical inspections of 30 individuals each, once a year for four years). In France, these programmes currently only involve VHS and IHN. The list of aquaculture zones and compartments certified free of VHS and/or IHN can be consulted on the MAAF website, at the following address: <http://agriculture.gouv.fr/maladies-des-animaux-aquatiques>.

Genetic monitoring

All strains of VHS and IHN virus isolated in France are collected by the NRL. The gene encoding the envelope glycoprotein of the VHS virus is systematically sequenced. More recently, the same procedure has been introduced for the IHN virus. A comparison of these sequences sometimes reveals multiple similarities that can be traced back to a common ancestor strain. These genetic studies are often useful in epidemiological investigations.

Health control measures

If an outbreak of a regulated disease is detected, health control measures are implemented (in compliance with Directive 2006/88/EC, transposed into French law by the decree of 4 November 2008). In the event of suspicion, the DDecPP or the DDAAF issues an APMS (prefectural monitoring order). If the infection is confirmed by an accredited laboratory and/or the NRL, the infected fish farm is placed under an APDI (prefectural declaration of infection), with measures for eliminating dead fish or those showing clinical symptoms and for draining, cleaning and disinfecting ponds. An epidemiological investigation is also carried out.

Regulatory References

Council Directive 2006/88/EC of 24 October 2006 on animal health requirements for aquaculture animals and products thereof, and on the prevention and control of certain diseases in aquatic animals. Please note that the rules for the application of this Directive have been adopted recently by the EU, detailing the procedures for surveillance and certification and proposing an update of the analytical methods used. These rules should enter into application in the course of 2016.

Order of 4 November 2008, on the health control measures applicable to animals and products in the aquaculture sector and the prevention of certain diseases in aquatic animals and the measures for combating these diseases.

Order of 8 June 2006 amended, on the animal health qualification or authorisation of primary production units, or businesses placing on the market products of animal origin or foodstuffs containing products of animal origin.

At European level, the review of surveillance data for 2013 (EURL data: <http://www.eurl-fish.eu/>) shows that cases of VHS and IHN are probably under-declared in a number of countries, with respective totals of 52 and 54 establishments (out of an overall number estimated at 8,896) considered infected. Same situation for KHV, with 50 fish hatcheries (out of 11,831) listed as infected on 31 December 2013.

For the countries of Northern Europe, the most problematic and common pathologies for 2014 were pancreatic disease (alphaviruses), Amoebic gill disease or AGD (pathogen = *Paramoeba perurans*) and Winter Ulcer Disease (*Moritella viscosa*). Sea lice continue to be a major problem. Continental Europe has been particularly affected by the aeromonases (*Aeromonas salmonicida*, *hydrophila*, etc.), Enteric Redmouth Disease (*Yersinia ruckeri*), flavobacteriosis (rainbow trout fry syndrome: *Flavobacterium psychrophilum*), and AGD, pathologies shared with the Mediterranean countries, which have also reported a number of cases of lactococcosis (*Lactococcus garvieae*) and nodaviriosis (a virus causing encephalopathy and retinopathy) in marine fish farms.

Acknowledgements

Thanks to Sylvie Perennec (from the DRAAF/SRAL of Basse Normandie) for her review of the paper and her corrections.

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