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FISH
DISEASES

SURVEILLANCE AND CONTROL METHODS FOR VIRAL HAEMORRHAGIC SEPTICAEMIA (VHS) AND INFECTIOUS HEMATOPOIETIC NECROSIS (IHN)



PART 1

SURVEILLANCE AND CONTROL METHODS FOR VIRAL HAEMORRHAGIC SEPTICAEMIA (VHS) AND INFECTIOUS HEMATOPOIETIC NECROSIS (IHN)**I. Requirements for surveillance and eradication programmes to obtain and maintain disease free-health statuses with regard to VHS and IHN and containment measures for those listed diseases****I.1. General requirements for health inspections and sampling for VHS and IHN:**

- (a) the health inspections and, where appropriate, the sampling shall be carried out during the period of the year when the water temperature is below 14 °C or whenever the water temperature is likely to reach its lowest annual points;
- (b) when targeted surveillance in wild populations is required in accordance with the second paragraph of point 2 of Part I of Annex V to Directive 2006/88/EC, the number and geographical distribution of sampling points shall be determined to obtain a reasonable coverage of the Member State, zone or compartment. The sampling points shall be representative of the different ecosystems where wild populations of susceptible species are located;
- (c) when farms or wild populations are to be subject to health inspections or sampled more than once per year, the intervals between the health inspections and between the collection of samples shall be at least 4 months and as long as possible, taking into account the temperature requirements provided for in point (a);
- (d) all production units, such as ponds, tanks and net cages, shall be subject to health inspections for the presence of dead, weak or abnormally behaving fish. Particular attention shall be paid to the water outlet area where weak fish tend to accumulate because of the water current;
- (e) fish of susceptible species to be collected as samples shall be selected as follows:
 - (i) if rainbow trout are present, only fish of that species shall be selected for sampling, except where other susceptible species are present which show typical signs of VHS or IHN; if rainbow trout are not present, the sample must be representative of all other susceptible species which are present;
 - (ii) if weak, abnormally behaving or freshly dead but not decomposed fish are present, such fish shall be selected; if more than one water source is utilised for fish production, fish representing all water sources shall be included in the sample;
 - (iii) the fish selected shall include fish collected in such a way that all parts of the farm, as well as all year classes, are proportionally represented in the sample.

I.2. Specific requirements to obtain disease-free health status (category I) with regard to VHS and IHN**I.2.1. Surveillance programmes:**

- (a) a Member State, zone or compartment that has Category III health status as referred to in Part B of Annex III to Directive 2006/88/EC with regard to VHS or IHN or both, may achieve Category I health status with regard to those listed diseases provided that all farms keeping susceptible species listed in Part II of Annex IV to that Directive within that Member State, zone or compartment comply with the requirements laid down in Annex V to that Directive and all those farms and, when required by the second paragraph of point 2 of Part I of Annex V thereto, sampling points in wild populations selected in accordance with that Part, have been subject to one of the following surveillance programmes:

- (i) model A — 2-year surveillance programme:

The farms or sampling points must have been subject to health inspections and sampled for a minimum period of two consecutive years as laid down in Table 1.A set out in Section II.

During that 2-year period, the testing of all samples using the diagnostic methods set out in point II.2 must have produced negative results for either VHS or IHN or both, and any suspicion of either VHS or IHN or both must have been ruled out in accordance with the sampling and diagnostic methods set out in point II.3;

- (ii) model B — 4-year surveillance programme with reduced sample size:

The farms or sampling points must have been subject to health inspections and sampled for a minimum period of four consecutive years as laid down in Table 1.B set out in Section II.

During that 4-year period, the testing of all samples using the diagnostic methods set out in point II.2 must have produced negative results for either VHS or IHN or both and any suspicion of either VHS or IHN or both must have been ruled out in accordance with the sampling and diagnostic methods set out in point II.3;

- (b) if during the implementation of the surveillance programme referred to in point (a), infection with either VHS or IHN or both is confirmed in a farm included in that surveillance programme, and therefore the farm's Category II health status has been withdrawn, that farm may immediately regain its Category II health status and continue the implementation of the surveillance programme to obtain disease-free status without implementing an eradication programme as set out in point I.2.2 provided that the farm complies with the following conditions:

- (i) it is a continental farm whose health status regarding either VHS or IHN or both is independent of the health status of aquatic animal populations in the surrounding natural waters as regards those listed diseases in accordance with point 3 of Part II of Annex V to Directive 2006/88/EC;
- (ii) it is emptied, cleansed, disinfected and fallowed; the duration of the fallowing period shall be at least 6 weeks;
- (iii) it has been restocked with fish sourced from Member States, zones or compartments with a Category I health status with regard to either VHS or IHN or both.

I.2.2. Eradication programmes

I.2.2.1. General requirements

A Member State, zone or a compartment with Category V health status with regard to either VHS or IHN or both may achieve Category I health status with regard to those listed diseases, provided that all farms keeping susceptible species listed in Part II of Annex IV to Directive 2006/88/EC within that Member State, zone or compartment have been subject to an eradication programme that complies with points (a) to (e):

- (a) the minimum control measures laid down in Section 4 of Chapter V of Directive 2006/88/EC must have been effectively applied and a containment area, as referred to in Article 32(b) of that Directive comprising a protection zone and surveillance zone, must have been established in the vicinity of the farm(s) officially declared infected with either VHS or IHN or both those listed diseases.

The containment area must have been defined on a case-by-case basis taking into account factors influencing the risks for the spread of the listed disease to farmed and wild fish, such as: the number, rate and distribution of the mortalities of fish on the farm infected with either VHS or IHN or both; the distance and density of neighbouring farms; the proximity to slaughterhouses; contact farms; species present at the farms; the farming practices applied in the affected farms and in the neighbouring farms to the affected farms; the hydrodynamic conditions and other factors of epidemiological significance identified.

For the establishment of the protection and surveillance zones, the following minimum requirements shall apply as regards the geographical demarcation of those zones:

- (i) a protection zone shall be established in the immediate vicinity of a farm officially declared infected with either VHS or IHN or both those listed disease and shall correspond to:
 - (1) in coastal areas: an area included in a circle with a radius of at least one tidal excursion or at least 5 km, whichever is larger, centred on the farm officially declared infected with either VHS or IHN or both, or an equivalent area determined according to appropriate hydrodynamic or epidemiological data;
 - (2) in inland areas: the entire water catchment area of the farm officially declared infected with VHS or IHN or both; the competent authority may limit the extension of the zone to parts of the water catchment area, or the area of the farm, provided that the prevention of the spread of either VHS or IHN or both is not compromised;
- (ii) a surveillance zone shall be established by the competent authority outside the protection zone and shall correspond to:
 - (1) in coastal areas: an area, surrounding the protection zone, of overlapping tidal excursion zones; or an area, surrounding the protection zone, and included in a circle of radius 10 km from the centre of the protection zone; or an equivalent area determined according to appropriate hydrodynamic or epidemiological data;
 - (2) in inland areas: as an extended area outside the established protection zone;
- (b) all farms keeping susceptible species listed in Part II of Annex IV to Directive 2006/88/EC within the protection zone not officially declared infected with either VHS or IHN or both shall be subject to an official investigation comprising at least the following elements:
 - (i) the collection of samples for testing of 10 fish, when clinical signs or *post-mortem* signs consistent with infection with either VHS or IHN or both are observed or minimum 30 fish, when clinical or *post-mortem* signs are not observed;
 - (ii) one health inspection: in those farms where the tests referred to in (i) have produced negative results; health inspections shall continue once per month during the period when the water temperature is below 14 °C, except when fish ponds or net cages are covered with ice, until the protection zone is withdrawn in accordance with point I.2.2.1(c);
- (c) all farms officially declared infected with either VHS or IHN or both shall be emptied, cleansed, disinfected and fallowed. The duration of the fallowing period shall be at least 6 weeks. When all farms officially declared infected within the same protection zone are emptied, at least 3 weeks of synchronised fallowing shall be carried out. This paragraph also applies to new farms officially declared infected during the implementation of the eradication programme.

When fallowing of the officially declared infected farms is carried out, the protection zones shall be converted into surveillance zones.

The competent authority may decide to require the emptying, cleansing, disinfection and fallowing of other farms within the established protection and surveillance zones. The length of the fallowing period for those farms shall be determined by the competent authority following a case-by-case risk evaluation;

- (d) all farms officially declared infected with either VHS or IHN or both those listed diseases and all other farms fallowed within the established protection and surveillance zones as referred to in point (c), shall be restocked with fish sourced from Member States, zones or compartments with a disease-free health status (Category I) with regard to either VHS or IHN or both.

Restocking shall only take place when all farms officially declared infected have been emptied, cleansed, disinfected and fallowed in accordance with point I.2.2.1(c);

- (e) all farms keeping susceptible species listed in Part II of Annex IV to Directive 2006/88/EC within the Member State, zone or compartment covered by the eradication programme and when surveillance in wild populations is required, sampling points selected in accordance with point I.1, shall subsequently be subject to the surveillance scheme laid down in point I.2.1.

I.2.2.2. Requirements for regaining disease-free status for continental compartments comprising one single farm previously being declared free of either IHN or VHS or both

A continental compartment comprising one single farm previously declared free of either VHS or IHN or both of those listed diseases, whose health status with regard to those listed diseases is independent of the surrounding natural waters in accordance with point 3 of Part II of Annex V to Directive 2006/88/EC, and whose Category I health status has been withdrawn in accordance with Article 53(3) of that Directive, may regain Category I health status immediately after the competent authority has confirmed that the following conditions have been complied with:

- (a) the officially confirmed infected farm with either VHS or IHN or both must have been emptied, cleansed, disinfected and fallowed; the duration of the fallowing period must be at least 6 weeks;
- (b) the officially confirmed infected farm with either VHS or IHN or both has been restocked with fish sourced from Member States, zones or compartments with a category I health status with regard to either VHS or IHN or both.

I.3. Specific requirements for the maintenance of disease-free health status (Category I) with regard to either VHS or IHN or both

When targeted surveillance is required in order to maintain Category I health status, as provided in Article 52 of Directive 2006/88/EC, all farms keeping susceptible species listed in Part II of Annex IV to that Directive within the Member State, zone or compartment concerned shall be subject to health inspection and fish shall be sampled in accordance with Table 1.C set out in Section II of this Part, taking into account the risk level of the farm for the contraction of either VHS or IHN or both of those listed diseases.

When determining the health inspection frequency for Category I health status compartments with regard to either VHS or IHN or both, which are placed in continental areas and where the health status regarding VHS or IHN is dependent on the health status of the aquatic animal populations in surrounding natural waters in accordance with point 2 of Part II of Annex V to Directive 2006/88/EC, the risk for the contraction of either VHS or IHN or both shall be considered as high.

Disease-free status shall only be maintained as long as all samples tested, using the diagnostic methods set out in point II.2., produce negative results for either VHS or IHN or both of those listed diseases and any suspicion of either VHS or IHN or both are ruled out in accordance with the diagnostic methods set out in point II.3.

I.4. Requirements for the lifting of containment measures provided for in Article 39 of Directive 2006/88/EC, namely the change from Category V to Category III health status

A Member State, zone or a compartment which has Category V health status with regard to either VHS or IHN or both may achieve Category III health status with regard to those listed diseases provided that:

- (a) the requirements set out in points I.2.2.1(a), (b) and (c) have been met. In case fallowing is not technically possible, the farms concerned shall be subject to an alternative measure which provides almost similar guarantee for extermination of either IHNV or VHSV or both from the environment of the farm;
- (b) all farms officially declared infected and all other farms fallowed/been subject to alternative measures in accordance with (a) within the established protection and surveillance zones have been restocked with fish sourced from Member States, zones or compartments with a Category I, II or III health status with regard to either VHS or IHN or both;

- (c) the restocking has only taken place after all farms officially declared infected, have been emptied, cleansed, disinfected and fallowed/been subject to alternative measures in accordance with (a).

II. Diagnostic and sampling methods

II.1. Organs to be sampled:

The tissue material to be examined is spleen, anterior kidney, and either heart or encephalon. When sampling broodstock ovarian or seminal fluid may also be examined.

In case of small fry, whole fish less than 4 cm long can be minced with sterile scissors or a scalpel after removal of the body behind the gut opening. If a sample consists of whole fish with a body length between 4 cm and 6 cm, the viscera including kidney shall be collected.

Organ pieces from a maximum of 10 fish may be pooled.

II.2. Diagnostic methods to obtain and maintain disease-free status for either VHS or IHN or both

The diagnostic method, in accordance with the approved diagnostic methods and procedures set out in point I. of Part 1 of Annex II, to achieve or to maintain disease-free status for VHS or IHN or both shall be either:

- (a) virus isolation in cell cultures followed by identification using enzyme-linked immunosorbent assay (ELISA), indirect fluorescent antibody test (IFAT), virus neutralisation test or real-time reverse transcriptase polymerase chain reaction (RT-qPCR); or
- (b) RT-qPCR.

II.3. Sampling and diagnostic methods to rule out or to confirm the presence of VHS or IHN

When a suspicion of either VHS or IHN or both is required to be confirmed or ruled out in accordance with Article 28 of Directive 2006/88/EC, the following inspection, sampling and testing procedures shall be complied with:

- (a) the farm under suspicion shall be subject to at least one health inspection and one sampling of 10 fish, when clinical signs or *post-mortem* signs consistent with infection with either VHS or IHN or both are observed or minimum 30 fish, when clinical or *post-mortem* signs are not observed. Samples shall be tested using one or more of the diagnostic methods set out in points (i) and (ii) in accordance with the detailed diagnostic methods and procedures as set out in Section II of Part 1 of Annex II:
- (i) conventional virus isolation in cell culture with subsequent immunochemical or molecular virus identification;
- (ii) virus detection by RT-qPCR;
- (iii) Other diagnostic techniques of proven similar efficacy such as Indirect fluorescent antibody test (IFAT), Enzyme-linked immuno-sorbent assay (ELISA), RT-PCR and Immunohistochemistry (IHC).
- (b) the presence of VHS shall be considered as confirmed, if one or more of those diagnostic methods are positive for VHSV. The presence of IHN shall be considered as confirmed, if one or more of those diagnostic methods are positive for IHNV. The confirmation of the first case of VHS or IHN in Member States, zones or compartments previously not infected shall be based on conventional virus isolation in cell culture or RT-qPCR;
- (c) Suspicion of either VHSV or IHNV or both may be ruled out, if cell cultivation or RT-qPCR tests reveal no further evidence of the presence of either VHSV or IHNV or both.

Table 1.A

Surveillance scheme for zones and compartments for the 2-year control period referred to in point I.2.1(a)(i) which precedes the achievement of disease-free status for VHS or IHN

Type of farm	Number of health inspections per year (2 years)	Number of samplings per year (2 years)	Number of fish in the sample ⁽¹⁾	
			Number of growing fish	Number of broodstock fish ⁽²⁾
(a) Farms with broodstock	2	2	50 (first inspection) 75 (second inspection)	30 (first or second inspection) 0 (first or second inspection)
(b) Farms with broodstock only	2	1	0	75 (first or second inspection)
(c) Farms without broodstock	2	2	75 ⁽³⁾ (first and second inspection)	0

Maximum number of fish per pool: 10

⁽¹⁾ The samples must be collected no sooner than 3 weeks after the transfer of the fish from fresh to saltwater.

⁽²⁾ Ovarian or seminal fluid of broodstock shall be collected at the time of maturation, in connection with stripping.

⁽³⁾ Samples must be taken from the number of fish that will ensure the detection of VHSV or IHNV with a 95 % confidence if the design prevalence is 5 %.

Table 1.B

Surveillance scheme with a reduced sample size for the 4-year control period referred to in point I.2.1(a)(ii) which precedes the achievement of disease-free status for VHS or IHN

Type of farm	Number of health inspections per year	Number of samplings per year	Number of fish in the sample ⁽¹⁾	
			Number of growing fish	Number of broodstock fish ⁽²⁾
First 2 years of the surveillance period				
(a) Farms with broodstock	2	1	0 (first inspection) 30 (second inspection)	0 (first inspection) 0 (second inspection)
(b) Farms with broodstock only	2	1	0	30 (first or second inspection)
(c) Farms without broodstock	2	1	30 ⁽³⁾ (first or second inspection)	0
Last 2 years of the surveillance period				
(a) Farms with broodstock	2	2	30 (first inspection) 0(second inspection)	0 (first inspection) 30 (second inspection)

Type of farm	Number of health inspections per year	Number of samplings per year	Number of fish in the sample ⁽¹⁾	
			Number of growing fish	Number of broodstock fish ⁽²⁾
(b) Farms with broodstock only	2	2		30 (first and second inspection)
(c) Farms without broodstock	2	2	30 ⁽³⁾ (first and second inspection)	

Maximum number of fish per pool: 10

⁽¹⁾ The samples must be collected no sooner than 3 weeks after the transfer of the fish from fresh to saltwater.

⁽²⁾ Ovarian or seminal fluid of broodstock shall be collected at the time of maturation, in connection with stripping.

⁽³⁾ Samples must be taken from the number of fish that will ensure detection of VHSV or IHNV with a 95 % confidence if the design prevalence is 10 %.

Table 1.C

Surveillance schemes for zones or compartments to maintain disease-free status for VHS or IHN as referred to in point I.3

Risk level	Number of health inspections	Number of fish in the sample ⁽³⁾
High	2 every year	30 ⁽¹⁾ ⁽²⁾
Medium	1 every year	30 ⁽¹⁾
Low	1 every 2 years	30 ⁽¹⁾

Maximum number of fish per pool: 10

⁽¹⁾ The samples must be collected no sooner than 3 weeks after the transfer of the fish from fresh to saltwater.

⁽²⁾ Samples must be taken from the number of fish that will ensure the detection of VHSV or IHNV with a 95 % confidence if the design prevalence is 10 %.

⁽³⁾ There shall be minimum one sample for every health inspection.