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DISEASES

## SURVEILLANCE AND CONTROL METHODS FOR KHV DISEASE

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## PART 2

**SURVEILLANCE AND CONTROL METHODS FOR KOI HERPESVIRUS DISEASE (KHVD)**

- I. **Requirements for surveillance and eradication programmes to obtain and maintain disease-free health status with regard to KHVD and to contain infection with koi herpesvirus (KHV)**
- I.1. General requirements

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 also be representative of the different ecosystems where the wild susceptible populations are located, namely, river systems and lakes.

Targeted surveillance shall rely on the regular monitoring of sites holding susceptible species. Sites shall be monitored when water temperatures have reached levels that are permissive for the development of the disease (> 15 °C) and no sooner than 2 weeks from the date such temperatures have been reached. Any diseased fish or fish showing abnormal behavior that are found on the site shall be sampled and tested.

Whenever possible, fish that have been kept for a prolonged time period at the virus permissive temperature range, namely 2 to 3 weeks at 15 °C to 26 °C, shall be sampled. The following approach may, however, be accepted:

- (a) to collect a sub-population at transfer from winter to summer ponds and hold the fish in the same water body as the summer pond until minimum temperature requirements have been obtained, or
- (b) to collect samples at harvesting or during other fish handling as part of normal management practices. If possible, samples shall be collected between 24 and 72 hours after such management practices to enhance the chance of KHV detection.

When farms or wild populations have to be subject to health inspections or sampled more than once per year, the intervals between the health inspections or collections of samples shall be as long as possible within the season when the water temperature is likely to reach its highest annual points without exceeding the limit of 28 °C.

All production units, such as ponds and tanks, must be subject to health inspections for the presence of dead, weak or abnormally behaving fish.

*Cyprinus carpio* and its hybrids, such as *Cyprinus carpio* × *Carassius auratus*, shall be collected when present in the farm.

The fish to be collected as samples shall be selected as follows:

- (i) if weak, abnormally behaving or freshly dead but not decomposed fish are present, such fish must be selected;
- (ii) if more than one water source is utilised for fish production, fish representing all water sources must be included for sampling;
- (iii) the fish selected must 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 achieve disease-free health status (Category I) with regard to KHVD

### I.2.1. Surveillance programmes

- (a) a Member State, zone or a compartment which has Category III health status with regard to KHVD may achieve Category I health status when all farms keeping susceptible species listed in Part II of Annex IV to Directive 2006/88/EC within that Member State, zone or compartment comply with the requirements for disease-free status set out in Annex V to that Directive and all those farms, and, when required by the second paragraph of point 2 of Part I of that Annex, 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 2.A set out in Section III.

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 KHV and any suspicion of KHVD must have been ruled out in accordance with the diagnostic methods set out in point III.2;

- (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 2.B set out in Section III.

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 KHV and any suspicion of KHVD must have been ruled out in accordance with the diagnostic methods set out in point III.2;

- (b) if during the implementation of the 4-year surveillance programme set out in point (a), infection with KHV is confirmed in a farm included in that surveillance programme, and therefore its Category II health status has been withdrawn, that farm may immediately regain the Category II health status and continue the implementation of the surveillance programme to obtain disease-free status without implementing an eradication programme as described in point I.2.2 provided that the farm complies with the following conditions:

- (i) it is a continental farm whose health status regarding KHVD is independent of the health status of aquatic animal populations in the surrounding natural water as regards that listed disease in accordance with point 3 of Part II of Annex V to Directive 2006/88/EC;
- (ii) it has been 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 KHVD.

## I.2.2. Eradication programmes

### I.2.2.1. General requirements

A Member State, zone or a compartment that has Category V health status with regard KHVD, may achieve Category I health status with regard to that listed disease when 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 at least to the following eradication programme:

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

The containment area must have been defined on a case-by-case basis taking into account factors influencing the risks for the spread of KHVD to farmed and wild fish, such as: the number, rate and distribution the mortalities of fish on the farm infected with KHV; the distance and density of neighbouring farms; the proximity to slaughterhouses; contact farms; the species present at the farms; the farming practices applied in the affected and neighbouring 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 KHV and shall correspond to the entire water catchment area of the farm officially declared infected with KHV; the competent authority may limit the extension of the zone to parts of the water catchment area provided that the prevention of the spread of KHVD is not compromised;
- (ii) a surveillance zone shall be established outside the protection zone and shall correspond to an extended area surrounding 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 KHV 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 KHVD are observed or 30 fish, when clinical or *post-mortem* signs are not observed;
  - (ii) one health inspection; in those farms, where the tests referred to in point III.2 have produced negative results; health inspections shall continue once per month during the season when the water temperature is likely to reach  $> 15^{\circ}\text{C}$  until the protection zone is withdrawn in accordance with point I.2.2.1(c);
- (c) all farms officially declared infected with KHV shall be emptied, cleansed, disinfected and fallowed. The duration of the fallowing period shall be at least 6 weeks. When all farms within the same protection zone officially declared infected have been 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 shall be determined by the competent authority following a case-by-case risk evaluation;

- (d) All farms officially declared infected with KHV and all other farms fallowed within the established protection and surveillance zones shall be restocked:
- (i) with fish sourced from Member States, zones or compartments with a Category I health status with regard to KHVD; or
  - (ii) for a transitional period until 31 December 2020, with fish from Member States, zones or compartments with an approved KHVD surveillance programme.

Restocking shall only take place when all farms officially declared infected with KHV 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 have subsequently been subject at least to the surveillance programme 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 declared free of KHVD

A continental compartment comprising one single farm that has Category I health status with regard to KHVD, whose health status with regard to KHVD 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 status has been withdrawn in accordance with Article 53(3) of that Directive, may regain Category I health status with regard to KHVD immediately after the competent authority has confirmed that it has complied with the following conditions:

- (a) it has been emptied, cleansed, disinfected and fallowed; the duration of the fallowing period must have been at least 6 weeks;
- (b) it has been restocked with fish sourced from Member States, zones or compartments with a Category I health status or compartments with an approved KHVD surveillance programme (Category II health status).

I.3. Specific requirements for the maintenance of Category I status with regard to KHVD

When targeted surveillance is required in order to maintain Category I health status, as provided for 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 sampled in accordance with Table 2.B set out in Section III of this Part, taking into account the risk level of the farm for the contraction of KHV.

The inspection frequency for health inspections of Category I compartments with regard to KHVD placed in continental areas and comprising one or more farms whose health status regarding KHVD is dependent on the health status for that listed disease of surrounding natural waters in accordance with point 2 of Part II of Annex V to Directive 2006/88/EC, shall be in accordance with the number set out for high risk level in that Table 2.C.

In Member States, zones or compartments in which the number of farms is limited and targeted surveillance on these farms does not provide sufficient epidemiological data, the surveillance schemes to maintain disease-free status shall include sampling points selected in accordance with the requirements set out in point I.1.

Those sampling points shall be inspected and sampled by rotation of 50 % of the sampling points each year. The sampling shall be carried out in accordance with Table 2.C set out in Section III. The samples shall be selected, prepared and examined as described in Section II and the laboratory examinations must be negative with regard to the presence of the agent of KHVD.

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 KHVD and any suspicion of KHVD must be ruled out in accordance with the diagnostic methods set out in point III.2.

I.4. Specific requirements for lifting the containment measures provided for in Article 39 of Directive 2006/88/EC to obtain Category III health status with regard to KHVD in Member States, compartments or zones that have Category V health status

A Member State, zone or a compartment that has Category V health status with regard to KHVD may achieve Category III health status with regard to that listed disease provided that:

- (a) the requirements set out in points I.2.2.1(a), (b) and(c) have been met. In case following is not technically possible, the farms concerned shall be subject to an alternative measure which provides almost similar guarantee for extermination of KHV from the environment of the farm;
- (b) all farms officially declared infected and all other farms followed/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 KHVD;
- (c) the restocking has only taken place when all farms officially declared infected have been emptied, cleansed, disinfected and followed/been subject to alternative measures in accordance with (a).

**II. Diagnostic and sampling methods for surveillance to obtain and maintain disease-free status with regard to KHVD**

II.1. Samples

The tissue material to be examined shall be parts of gill and kidney. Organ pieces from a maximum of two fish may be pooled.

II.2. Diagnostic methods for the surveillance to obtain and maintain disease-free status with regard to KHVD

The diagnostic method to achieve or to maintain disease-free status for KHVD shall be Real-time PCR (qPCR) in accordance with the detailed diagnostic methods and procedures as set out in point II of Part 2 of Annex II.

### III. Diagnostic and sampling methods for official investigations for confirmation or to rule out a suspicion of KHVD

#### III.1. Samples

The tissue material to be examined shall be parts of gill and kidney. Organ pieces from a maximum of two fish may be pooled.

#### III.2. Official investigation and diagnostic methods to rule out and to confirm the presence of infection with KHV

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

(a) the official investigation shall include at least one health inspection and one sampling of 10 fish, when clinical signs or post-mortem signs consistent with infection with KHV are observed or 30 fish when clinical or post-mortem signs are not observed. Samples shall be tested using the diagnostic method set out in point (b) in accordance with the detailed diagnostic methods and procedures set out in point II of Part 2 of Annex II;

(b) the presence of the infection with KHV shall be considered as confirmed if KHV is detected by PCR;

suspicion of KHVD may be ruled out, if this test reveals no further evidence of the presence of KHV.

Table 2.A

#### Surveillance scheme for zones and compartments for the 2-year control period which precedes the achievement of disease-free status for KHVD as referred to in point I.2.1

		Number of clinical inspections per year (2 years)	Number of laboratory examinations per year (2 years)	Number of fish in the sample
Farms/sampling sites	First 2 years of the surveillance period	2	2	75 <sup>(1)</sup>
	Maximum number of fish per pool: 2			

<sup>(1)</sup> Samples must be taken from the number of fish that will ensure the detection of KHV with a 95 % confidence if the design prevalence is 5 %.

Table 2.B

#### Surveillance scheme for zones and compartments for the 4-year control period which precedes the achievement of disease-free status for KHVD as referred to in point I.2.1

		Number of clinical inspections per year	Number of laboratory examinations per year	Number of fish in the sample
Farms/sampling sites	First 2 years of the surveillance period	1	1	30
Farms/sampling sites	Last 2 years of the surveillance period	2	2	30
	Maximum number of fish per pool: 2			

Table 2.C

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

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

Maximum number of fish per pool: 2

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Table 2.D

**Surveillance scheme to maintain KHVD disease-free status in Member States, zones or compartments where the number of farms is limited and targeted surveillance on these farms does not provide sufficient epidemiological data as referred to in point I.3**

	Number of clinical inspections per year	Number of laboratory examinations per year	Number of fish in the sample
Sampling points	1 every 2 years	1 every 2 years	30

Maximum number of fish per pool: 2

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