Japan’s comments on the Report of the meeting of the OIE Aquatic Animal Health Standards Commission in September 2020

(Annex15) Infection with koi herpesvirus

Proposed amendments and comments on 1: Scope (~~Deletion~~)

Infection with koi herpesvirus means infection with ~~all genotypes of~~ the pathogenic agent cyprinid herpesvirus-3 (CyHV-3), of the Genus Cyprinivirus in the Family Alloherpesviridae (Haramoto et al., 2007; Waltzek et al., 2009).

(Rationale)

Since the phrase of "all genotypes of" may lead to misunderstanding that the pathogenic agent includes the variants mentioned in Engelsma et al.(2013), it should be removed to avoid unnecessary confusion.

Proposed amendments and comments on (3.6. Pooling of samples) (Insertion/~~Deletion~~)

The effect of pooling on diagnostic sensitivity has not been evaluated, therefore, larger fish ~~should~~ is recommended to be processed and tested individually. it is possible to pool only when multiple individuals in the same group are examined, with taking the diagnostic sensitivity into consideration. Small life stages such as fry or specimens up to 0.5 g, can be pooled to obtain the minimum amount of material for virus isolation or molecular detection.

(Rationale)

It’s extreme to stipulate that larger fish should be individually inspected, even though the effects of the pool have not been assessed. Given the pathogenic characteristics of the KHV, it’s unlikely that dilution with a pool will reduce sensitivity, as infection is expected to spread quickly from individual to group. Therefore, Japan considers that it should be allowed to pool in the same group.

Proposed amendments and comments onTable 4.1. :OIE recommended diagnostic methods and their level of validation for surveillance of healthy animals and investigation of clinically affected animals (Insertion)

Method	A. Surveillance of apparently healthy animals				2. Presumptive diagnosis of clinically affected animals				3. Confirmatory diagnosis[1] of a suspect result from surveillance or presumptive diagnosis			
	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV
Histopathology						++	++	1				
Cell or artificial media culture						++	++	1				
Real-time PCR	++	++	++	1	++	++	++	1	++	++	++	1
Conventional PCR	++	++	++	1	++	+++	+++	1	++	++	++	1
Conventional nested PCR	++	++	++	1	+++	+++	+++	1	++	++	++	1
Amplicon sequencing[3]									+++	+++	+++	1
In-situ hybridisation												
Bioassay												
LAMP						+++	+++	1				
IFAT						+	+	1				
Serology (ELISA)			++	1			++	1				

(Rationale)

- Japan would like to know why the conventional PCR is rated as not appropriate~~s~~ for Surveillance of apparently healthy animals in this draft because it has been rated as B in the previous manual. Otherwise, it should be evaluated as ++ as before, at least until the basis for the evaluation is shown.
- Japan also would like to know why Serology (ELISA) is rated as not appropriate~~s~~ for Surveillance of apparently healthy animals and Presumptive diagnosis of clinically affected animals for adults in this draft because they have been rated as B in the previous manual~~s~~. Otherwise, it should be evaluated as ++, as before, at least until the basis for the evaluation is shown.

Comments on (4.3. Cell or artificial media culture for virus isolation)

Cell lines should be monitored to ensure that susceptibility to targeted pathogens has not changed.
Diagnosis of infection with KHV in clinically affected fish can be achieved by virus isolation in cell culture. However, the virus is isolated in only a limited number of cell lines which can be difficult to handle. Also, cell culture isolation is not as sensitive as the published PCR-based methods to detect KHV DNA and is not considered to be a reliable diagnostic method for KHV (Haenen et al., 2004).
Cell line to be used: KF-1, KFC or CCB
Use the procedure described in Chapter 2.3.0 General information (on diseases of fish), Section A.2.2.2.
Confirmatory identification
The most reliable method for confirmatory identification of a CPE is by PCR, followed by sequence analysis of the PCR product. The PCR methods recommended for identification of KHV are the same methods recommended for direct detection in fish tissues (Section 4.3.1.2.3 below). For final confirmation, PCR products of the correct size should be identified as KHV in origin by sequence analysis.
i) Using a suitable DNA extraction kit or reagent, extract DNA from a sample of the virus culture that includes both cellular and supernatant material.
ii) Extracted DNA is then amplified using the PCR protocols described below. Amplified PCR products may then be excised from the gel and sequenced as described in Section 4.3.1.2.3.

(Comments)
Japan would like to request to show the reference pertaining to the listing of KFC i as a cell line for virus isolation because it is not a familiar cell line. If KFC is an appropriate cell line, It should also be added to the list in Chapter 2.3.0, General Information B. 1.1 Fish cell line.

Proposed amendments and comments on 4.3.3: *In-situ* hybridisation) (Insertion/~~Deletion~~)

Engelsma et al. (2013) reported that the published single-round PCR methods traditionally thought to be the most sensitive for detection of KHV DNA in fresh tissue samples fail to detect some KHV genotypes in clinically affected fish. Therefore, the assay described by Engelsma et al. (2013) ~~is highly recommended~~ can be used when detecting KHV variants. ~~By extending the number of cycles to 50 or using the nested second round of amplification the assay may also be suitable to detect virus in sub-clinical carriers.~~ However, the pathogenicity of this KHV variant has not yet been confirmed. This method and other commonly used PCR protocols are shown in Table 4.4.3.

(Rationale)

a) Since the variants 1-3 with the genetic sequences detected by Engelsma et al. (2013) has not been isolated in cells and their infectivity to fish have not been proven, it is not clear whether or not they are pathogenic viruses capable of transmission. Japan considers that It should be interpreted that these variants are included in pathogens only after they are isolated in cells and analysed for their properties, including pathogenicity, and it is proven that these are pathogenic viruses.

   Therefore, the nested PCR of Engelsma et al. (2013) should only be introduced as a method that can detect variants 1-3, and it should be mentioned that the pathogenicity of these variants has not yet been confirmed. Japan considers that before recommending the nested PCR of Engelsma et al. (2013), it is necessary to compare and verify the testing accuracy between this method and each existing method.

b) Although the draft describes " By extending the number of cycles to 50 may also be suitable", it is not mentioned in the reference by Enelsma et al. and is generally not a good method because it is likely to cause the appearance of non-specific bands and because some commercial enzymes are thought to be inactivated by 50 thermal cycles. Japan considers that thermal cycling should not exceed 40 cycles in general. The number of cycles should be stated after validation.

Proposed amendments and comments on 5: Test(s) recommended for surveillance to demonstrate disease freedom in apparently healthy populations (Insertion/~~Deletion~~)

There are no well validated methods that are currently recommended for testing healthy populations of susceptible fish for declaration of freedom from infection with KHV; there is increasing evidence that the published real-time PCR assays may fail to detect all genotypes of KHV. Therefore, conventional nested PCR assays described by Engelsma et al. (2013) which will detect all known KHV genotypes ~~is~~ may be currently recommended for surveillance to demonstrate freedom in apparently health

(Rationale)

Since the nested PCR described by Engelsma et al. is not a popular method, Japan believes that a comparative verification of detection sensitivity and reaction specificity between the nested PCR and each existing method. is required to recommend the nested PCR. Until then, the evaluation of the conventional PCR should be ++. Therefore, Japan considers that nested PCR "may be currently recommended" but not "is currently recommended" for proof of freedom.

(Annex16) General Information

Comments on 1.3: Specifications according to clinical status

For diagnosis of clinical infection for most viruses, appropriate organs to sample include anterior/mid kidney, spleen and either heart or encephalon; for fry whole fish or entire viscera may be used. For koi herpesvirus, gill and gut should be sampled; for epizootic ulcerative syndrome, skin or muscle; and for *Gyrodactylus salaris*, whole fish or fins should be examined. Samples from ten clinically diseased fish should be sufficient for the pathogen test(s) for each epidemiological unit. For detecting subclinical infections or for targeted surveillance, refer to individual disease chapters of the Aquatic Manual and chapter 1.4 of the OIE Aquatic Code.

(Comment)

The organs described in the General Information are inconsistent with the ones described in 3.2 Selection of Annex15, which states that appropriate organs for sampling are the gills, kidneys and spleen.

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（6. TEXTS FOR MEMBER INFORMATION）

Proposed comments on 6.3: Infection with Carp edema virus (CEV)

The Commission considered the comments received, including advice from some countries that infection with CEV had already emerged within their countries and that it has been detected for some years. The Commission agreed that it would continue to monitor the situation with CEV and requested that countries report infection with CEV as an emerging disease in accordance with Article 1.1.4 of the Aquatic Code.

Comments：

Japan requests the OIE to share the records of meetings where CEV were judged as an emerging disease and scientific justification of the judgement such as scientific papers.

According to the information from NACA between 2017 and 2019, only 8 cases of CEV occurrences have been reported by 3 countries in Asia. In addition, recent mortality in each case is lower than before. Objective data for demonstrating a) its spread to a new geographic area are insufficient, though there is a possibility that CEV is b) a newly recognized or suspected pathogenic agent. At present, Japan does not recognize that there is enough information to judge CEV has a significant impact on aquatic animal or public health, although some countries suggest that CEV can make a severe impact on farms.

Based on the above, Japan requests the OIE to discuss again whether or not CEV meets the definition for the emerging disease or listed disease, taking into account descriptions in scientific papers and the reports submitted o NACA and WAHIS as well as the outbreak and damage in each country.

（Reference）EMERGING DISEASE from OIE glossary

means a disease, other than listed diseases, which has a significant impact on aquatic animal or public health resulting from:

a) a change of known pathogenic agent or its spread to a new geographic area or species; or

b) a newly recognized or suspected pathogenic agent.