Annex 7. Item 6.4. – Assessment of infection with all genogroups of the virus species infectious spleen and kidney necrosis virus for listing in the *Aquatic Code*

**Assessment of infection with all genogroups of the virus species infectious spleen and kidney necrosis virus (ISKNV) for listing in the WOAH *Aquatic Animal Health Code***

**Assessment summary**

1. The Aquatic Animal Health Standards Commission assessed the virus species *Infectious spleen and kidney necrosis virus*, including its three genogroups red sea bream iridovirus (RSIV), infectious spleen and kidney necrosis virus (ISKNV), and turbot reddish body iridovirus (TRBIV) against the criteria for listing aquatic animal diseases in Article 1.2.2. of the *Aquatic Code.*
2. The Aquatic Animals Commission agreed that the RSIV genogroup (currently listed in the *Aquatic Code*), as well as the two genogroups ISKNV and TRBIV meet the listing criteria 1, 2, 3, and 4b (see Table 1 below).
3. The Aquatic Animals Commission noted that the three genogroups have overlapping susceptible species, similar epidemiology, and similar diagnostic methods. The Commission agreed that the proposed listed disease should be named “infection with all genogroups of the virus species ISKNV”. Infection with all genogroups of the virus species ISKNV would be defined to include the genogroups ISKNV, RSIV and TRBIV but would exclude the other recognized species of *Megalocytivirus*, *Scale drop disease virus*.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Listing criteria | | | | | | Conclusion |
| 1 | 2 | 3 | 4a | 4b | 4c |
| Infection with all genogroups of the viral species ISKNV | + | + | + | NA | + | - | The disease meets the criteria for listing. |

NA = not applicable.

**Listing Criteria (Chapter 1.2. of the *Aquatic Code*)**

The criteria for the inclusion of a disease in the WOAH list are as follows:

1. International spread of the pathogenic agent (via aquatic animals, aquatic animal products, vectors or fomites) is likely.

AND

1. At least one country may demonstrate country or zone freedom from the disease in susceptible aquatic animals, based on provisions of Chapter 1.4.

AND

1. A precise case definition is available and a reliable means of detection and diagnosis exists.

AND

4a. Natural transmission to humans has been proven, and human infection is associated with severe consequences.

OR

4b. The disease has been shown to affect the health of cultured aquatic animals at the level of a country or a zone resulting in significant consequences e.g. production losses, morbidity or mortality at a zone or country level.

OR

4c. The disease has been shown to, or scientific evidence indicates that it would affect the health of wild resulting in significant consequences e.g. morbidity or mortality at a population level, reduced productivity or ecological impacts.

**Background**

*Megalocytivirus* is one of seven genera of the family *Iridoviridae* and is classified within the subfamily Alphairidovirinae together with the genera *Ranavirus* and *Lymphocystivirus* (Chinchar *et al*., 2017; Chinchar *et al*., 2020). Megalocytiviruses are distinguished from ranaviruses and lymphocystiviruses by their ability to trigger marked cell enlargement in infected tissues and by sequence analysis of key viral genes (Chinchar *et al*., 2017). Megalocytiviruses are the aetiological agents of severe disease associated with high mortality in a range of marine and freshwater finfish species (Kurita & Nakajima, 2012; Hick *et al*., 2016).

The ICTV recognises two species of *Megalocytivirus*: *Infectious spleen and kidney necrosis virus* (ISKNV) and *Scale drop disease virus* (SDDV) (Chinchar *et al*., 2017). SDDV is genetically and epidemiologically distinct from the species ISKNV and is not considered further in this assessment.

Within the species ISKNV, three genogroups have been recognised: ISKNV, RSIV and TRBIV (Song *et al.*, 2008). However, it remains to be resolved whether these genogroups represent distinct species, or strains of a single species (Chinchar *et al*., 2017). Megalocytiviruses have been given numerous unique names based on the species that they were detected in; however, all variants of the species ISKNV that have had their genomes analysed are placed within the three genogroups: ISKNV, RSIV and TRBIV (Chinchar *et al*., 2017). Each genogroup is further subdivided into two clades (Koda *et al*. 2018, 2019, 2023; Fusianto *et al*. 2023).

The name ISKNV is used for one of two recognised species of *Megalocytivirus* and also for one of the three genogroups within the ISKNV species. When used within this document, “ISKNV genogroup” refers to the genogroup ISKNV and “the species ISKNV” is used whenever referring to the species ISKNV.

Infection with red sea bream iridovirus (RSIV) was first listed by WOAH in the 2003 *Aquatic Animal Health Code*0F0F[[1]](#footnote-2) and remains listed in the 2023 *Aquatic Code*. Disease caused by RSIV was first detected in cultured red sea bream (*Pagrus major*) in Japan in 1990 (Inouye *et al*., 1992). RSIV has been detected principally from marine fish. Species currently listed as susceptible to infection with RSIV in the WOAH *Aquatic Code* include:1F1F[[2]](#footnote-3): red sea bream (*Pagrus major*), yellowtail (*Seriola quinqueradiata*), amberjack (*Seriola dumerili*), sea bass (*Lateolabrax* sp.), Asian sea bass (*Lates calcarifer*), albacore (*Thunnus thynnus*), Japanese parrotfish (*Oplegnathus fasciatus*), striped jack (*Caranx delicatissimus*), mandarin fish (*Siniperca chuatsi*), red drum (*Sciaenops ocellatus*), mullet (*Mugil cephalus*) and groupers (*Epinephelus spp.*).

The ISKNV genogroup is not currently listed in the WOAH *Aquatic Code*. Virions morphologically consistent with iridoviruses and presenting enlarged cells with inclusion bodies consistent with megalocytiviruses have been reported in species of freshwater fish since the late 1980s and 1990s (e.g. Armstrong & Ferguson, 1989; Anderson *et al.*, 1993). ISKNV genogroup has been detected in archival ornamental fish samples from as early as 1996 (Go *et al*., 2006; Go *et al*., 2016; Becker *et al*., 2022). Infectious spleen and kidney necrosis disease was described from mandarin fish (*Siniperca chuatsi*; He *et al*., 2000; He *et al*., 2002) and in 2001 the genome of ISKNV genogroup was analysed and found to be genetically similar to RSIV (He *et al*., 2001). ISKNV genogroup has been detected from numerous freshwater fish species, including many associated with ornamental fish trade (see review by Johan & Zainathan, 2020; Becker *et al*., 2022). This genogroup has been reported from numerous species of ornamental fish that have been traded internationally (see Rimmer *et al*., 2015). ISKNV genogroup has also been reported as a cause of mass mortality in species important for human consumption (e.g. Subramaniam *et al.*, 2016; Ramírez-Paredes *et al.*, 2020; Fusianto *et al*., 2021).

The TRBIV genogroup is not currently listed in the WOAH *Aquatic* *Code*. TRBIV was first described as causing disease in turbot, *Scophthalmus maximus* (Shi *et al.,* 2004). TRBIV has been known to principally cause disease in flatfishes in China and Korea (e.g. Shi *et al.,* 2004; Do *et al.,* 2005) but it has also been detected in other species including in the ornamental fish trade (Go *et al*., 2016; Koda *et al*., 2018). TRBIV has also caused disease in other economically important farmed fish species such as Asian sea perch (*Lates calcarifer*) (Tsai *et al.*, 2020) and barred knifejaw (*Oplegnathus fasciatus*) (Huang *et al.*, 2011).

The Aquatic Animals Commission previously proposed an approach to differentiating pathogen strains (refer to the Commission’s [February](https://www.woah.org/fileadmin/Home/eng/Internationa_Standard_Setting/docs/pdf/Aquatic_Commission/A_AAC_Feb_2011.pdf) and [October 2011](https://www.woah.org/fileadmin/Home/eng/Internationa_Standard_Setting/docs/pdf/A_AAC_October_2011.pdf) meeting reports). Three main criteria were considered for the applicability of pathogen strain differentiation in the standards of the *Aquatic* *Code* and *Aquatic* *Manual*: 1) variants of the pathogen are clearly recognized in the scientific literature and have different disease characteristics; 2) there are robust methods for consistently differentiating the variants; and 3) there is, or there is potential for, different management of variants within or between countries. In the case of the species ISKNV, RSIV was listed prior to research that defined the 3 genogroups within the species ISKNV, and the genetic and epidemiological relationships among them. Given the precedent of infection with RSIV having been listed, but not the ISKNV and TRBIV genogroups, this assessment presents information for each of these three genogroups, despite the three genogroups, being proposed for listing collectively as the species ISKNV.

**Assessment against listing criteria**

**Criterion No. 1.** International spread of the pathogenic agent (via aquatic animals, aquatic animal products, vectors or fomites) is likely.

*Assessment*

The species ISKNV can be transmitted horizontally via water and is known to remain viable in frozen host tissues. The likelihood of transmission is expected to be greater for trade in live fish but is also possible in aquatic animal products, particularly if not eviscerated.

Numerous marine and freshwater species are susceptible to the species ISKNV and are traded internationally, either as live aquatic animals (for human consumption, aquaculture or for ornamental purposes) or as aquatic animal products.

RSIV has been detected in several countries in Asia where it has been associated with disease in species of farmed marine fish (Kurita & Nakajima, 2012). Some susceptible species are traded live for human consumption (e.g. red sea bream, groupers), others are traded as aquatic animal products.

ISKNV genogroup has been detected in numerous species traded as ornamental fish and the ornamental fish trade has been implicated in disease spread and outbreaks (e.g. Jeong *et al*., 2008; Johan & Zainathan, 2020). Infected ornamental fish may not present clinical signs (e.g. Subramaniam *et al*., 2014; Rimmer *et al*., 2015) and as such may act as carriers of the virus. ISKNV genogroup has also been detected in important farmed species for human consumption that are traded internationally, such as tilapia (Ramírez-Paredes *et al*., 2020). ISKNV genogroup has also been detected in unprocessed fish used for aquaculture feed (Lajimin *et al*., 2015) suggesting that fish traded for aquaculture feed or bait may present a pathway. Transmission from freshwater finfish species to marine finfish species has been demonstrated by direct inoculation and cohabitation (Jeong *et al*., 2008b; Go & Whittington, 2019).

TRBIV is known to occur in several species that are important for international trade (e.g. turbot, flounder, Asian sea bass), including live trade or as aquatic animal products. Phylogenetic analysis indicates that there has been recent international spread of TRBIV (Tsai *et al*., 2020).

Variants of the species ISKNV have been detected in numerous species of marine and freshwater species that are traded internationally. Each of the three genogroups has been detected in traded commodities and there is evidence of international spread associated with trade.

*Conclusion*

The criterion is met.

**Criterion No. 2.** At least one country may demonstrate country or zone freedom from the disease in susceptible aquatic animals, based on provisions of Chapter 1.4.

*Assessment*

Infection with RSIV has been notifiable to the WOAH since 2003. Several countries continue to report that RSIV has never been reported from their territory (refer to WOAH World Animal Health Information System) and it is likely that some of these countries could demonstrate country freedom.

ISKNV genogroup has been reported from numerous fish species traded through the ornamental fish trade and it is likely that this genogroup is widespread through ornamental fish supply chains. However, some countries maintain *basic biosecurity measures*2F2F[[3]](#footnote-4) for ISKNV genogroup and may be able to demonstrate freedom. Further, PCR assays used in surveillance for RSIV would also detect ISKNV genogroup, providing evidence of freedom from ISKNV genogroup.

TRBIV has been primarily detected in farmed flatfish from China and Korea but has also been detected in ornamental fish and in farmed Asian sea bass. PCR assays recommended in the WOAH *Aquatic Manual* chapter for RSIV may not be inclusive of TRBIV resulting in a lower confidence in the distribution of TRBIV. However, given TRBIV has demonstrated pathogenicity in farmed populations of several species, it is likely that TRBIV would be detected in those species if it had occurred. Although there is less certainty regarding the distribution of TRBIV, it seems likely that at least one country could claim freedom at the level of country or zone.

*Conclusion*

The criterion is met.

**Criterion No. 3.** A precise case definition is available and a reliable means of detection and diagnosis exists.

*Assessment*

Case definitions for suspicion and confirmation of infection with RSIV are available in the WOAH *Aquatic Manual*. As some PCR assays for RSIV (and some other methods, e.g. histopathology), are inclusive of ISKNV genogroup, the case definitions could be easily adapted to include ISKNV genogroup. Kawato *et al.* (2021) compared the analytical performance of four real-time PCR methods for the detection of megalocytiviruses (excluding SDDV) and found that three of the four assays detected ISKNV, RSIV, and TRBIV genogroups. Kim *et al.* (2022) reported on the performance of a real-time PCR assay with inclusivity for RSIV, ISKNV and TRBIV genogroups. There are sufficient diagnostic tools available to detect the species ISKNV and to construct case definitions inclusive of the three genogroups.

*Conclusion*

Criterion is met.

**Criterion No. 4a**Natural transmission to humans has been proven, and human infection is associated with severe consequences.

*Assessment*

There is no evidence of transmission to humans.

*Conclusion*

Criterion not applicable.

**Criterion No. 4b** The disease has been shown to affect the health of cultured aquatic animals at the level of a country or a zone resulting in significant consequences e.g. production losses, morbidity or mortality at a zone or country level.

*Assessment*

RSIV has caused mass mortalities in cultured fish populations. The disease was first detected in red sea bream in Japan with affected fish becoming lethargic, exhibiting severe anaemia, petechiae of the gills, and enlargement of the spleen (Inouye *et al*., 1992; Jung *et al*., 1997; Nakajima & Maeno, 1998). RSIV has been reported to cause production losses, morbidity and mortality in many other species (e.g. Chao *et al.*, 2004; Chen *et al.*, 2003; Girisha *et al.*, 2020; Ni *et al.*, 2021; Sumithra *et al.*, 2022).

ISKNV genogroup has been associated with numerous cases of disease in ornamental fish (see review by Johan & Zainathan*,* 2020; Becker *et al*., 2022). ISKNV genogroup has also been associated with high mortalities in important species farmed for human consumption; for example, in Asian sea bass (Dong *et al.,* 2017; Kerddee *et al*., 2021), Tilapia (e.g. Figueiredo *et al*., 2021; Ramírez-Paredes *et al*., 2021) and groupers (e.g. Chao *et al.*, 2004; Huang *et al.*, 2020; Fusianto *et al*., 2021).

TRBIV has caused disease and high mortality in turbot aquaculture in China (e.g. Shi *et al.*, 2010). Mortalities of up to 90% have occurred in Asian sea bass farms in Taiwan (Tsai *et al.,* 2020).

*Conclusion*

Criterion is met.

**Criterion No. 4c** The disease has been shown to, or scientific evidence indicates that it would affect the health of wild resulting in significant consequences e.g. morbidity or mortality at a population level, reduced productivity or ecological impacts.

*Assessment*

There is limited information on the occurrence of RSIV, ISKNV or TRBIV genogroups in wild fish populations and their consequences such as morbidity, mortality or ecological impacts. ISKNV genogroup has been reported as the cause of a mass mortality event in a population of wild cichlids in India (Swaminathan *et al*., 2022), but has also been detected in many apparently healthy wild fish from a diverse range of fish species (Wang *et al*., 2007).

*Conclusion*

Criterion is not met.

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1. RSIV was included in the *Aquatic Code* prior to 2003 as an “other disease of significance”. [↑](#footnote-ref-2)
2. Note that the species listed as susceptible to infection with RSIV in accordance with Chapter 1.5. of the *Aquatic Code* has not been revised based on the recommendations of the *ad hoc* Group. [↑](#footnote-ref-3)
3. Basic Biosecurity conditions are defined in article 1.4.6. of the *Aquatic Code* and include requirements for an early detection system (as described in Article 1.4.7.) and measures to prevent the introduction of the pathogenic agent. [↑](#footnote-ref-4)