Organisation Mondiale de la Santé Animale | World Organisation for Animal Health | Organización Mundial de Sanidad Animal

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INFORME DE LA REUNIÓN DE LA COMISIÓN DE NORMAS SANITARIAS PARA LOS ANIMALES ACUÁTICOS DE LA OIE

París, 19–26 de febrero de 2020

La epidemia de COVID-19 ha exigido revisar las modalidades de participación de los Miembros en reuniones internacionales, en particular en la 88.ª Sesión General de la Asamblea Mundial de Delegados de la OIE. En este contexto, el Consejo de la OIE celebró dos reuniones extraordinarias, en abril y mayo de 2020 y decidió, en acuerdo con la directora general, que la 88.ª Sesión General de mayo de 2020 se postergaría hasta 2021 y propuso procedimientos alternativos para tratar asuntos institucionales y administrativos esenciales.

En consecuencia, en 2020 no se propondrá la adopción de ningún capítulo nuevo o modificado en el *Código Sanitario para los Animales Acuáticos*, el *Código Sanitario para los Animales Terrestres*, el *Manual de Pruebas de Diagnóstico para los Animales Acuáticos* y el *Manual de las Pruebas de Diagnóstico y de las Vacunas para los Animales Terrestres*. Los capítulos que se proponían para adopción en 2020 se propondrán para adopción en 2021.

Con el fin de garantizar un enfoque coherente entre las tres comisiones especializadas que presentan normas a la Asamblea Mundial, la OIE ha decidido lo siguiente:

1. Todos los textos relevantes que se habían propuesto para adopción en mayo de 2020 se difundirán en el informe de febrero de 2020 de la correspondiente comisión especializada, sabiendo que la adopción se postergará hasta mayo de 2021 y que estarán abiertos para una ronda de comentarios.
2. Sólo se considerarán comentarios sustanciales que no se hayan presentado anteriormente.
3. El plazo para transmitir comentarios sobre los anexos de la Comisión para los Animales Acuáticos es el 10 de julio de 2020.
4. Cada Comisión examinará los comentarios en sus reuniones de septiembre de 2020 o de febrero de 2021, difundirá su trabajo y facilitará así que se progrese en otros asuntos.
5. Los textos (que incorporen las revisiones resultado de este proceso) se incluirán en los informes de febrero de 2021 de cada Comisión y se propondrán para adopción en mayo de 2021.
6. Este proceso no altera el procedimiento regular que se aplica a otros capítulos que circulan para comentario.

La Comisión de Normas Sanitarias de la OIE para los Animales Acuáticos (en lo sucesivo, Comisión para los Animales Acuáticos) se reunió en la sede de la Organización, en París, del 19 al 26 de febrero de 2020. La lista de participantes figura en el Anexo 1.

La Comisión para los Animales Acuáticos agradece a los siguientes Miembros por el envío de sus comentarios sobre los proyectos de texto para el *Código Sanitario para los Animales Acuáticos* (*Código Acuático*) y el *Manual de las Pruebas de Diagnóstico para los Animales Acuáticos* (*Manual Acuático*) que circularon tras su reunión de septiembre de 2019: Argentina, Australia, Canadá, Corea, Chile, China (Rep. Pop.), Cuba, Ecuador, Estados Unidos de América, Japón, Nicaragua, Noruega, Nueva Caledonia, Nueva Zelanda, Perú, Singapur, Tailandia, Taipéi Chino, los Estados Miembros de la Unión Europea (UE) y la Unión Africana Oficina Interafricana de Recursos Pecuarios (AU-IBAR) en nombre de los 54 Miembros africanos de la OIE.

La Comisión para los Animales Acuáticos examinó todos los comentarios presentados a tiempo y acompañados de una justificación. Las enmiendas realizadas por la Comisión a los proyectos de textos cuando fuera relevante se señalan del modo habitual, mediante "doble subrayado" y "~~tachado~~". En los anexos, los cambios propuestos en esta reunión se muestran con un fondo de color para distinguirlos de los realizados anteriormente. La Comisión no tuvo en cuenta los comentarios sin justificación o difíciles de interpretar.

Igualmente, en la preparación de sus comentarios, invita a los Miembros a consultar información pertinente en informes anteriores de la Comisión y de grupos *ad hoc* que presentan información de gran interés, especialmente cuando se trata de temas de larga data. Estos informes están disponibles en el sitio web de la OIE.

El índice presentado a continuación enumera los temas del orden del día con un enlace directo a los textos correspondientes. Los Miembros deben tomar nota de que los textos de los **Anexos 2 a 7** y **11 a 14**, que se presentaban para adopción en mayo de 2020, se presentarán para adopción en mayo de 2021 y pasarán por una ronda adicional de comentarios. Como estos textos ya se sometieron a un análisis exhaustivo, se solicita a los Miembros remitir observaciones sustanciales que no se hayan considerado previamente. Los Anexos **8 a 9 y 15 a 16** se presentan para comentario de los Miembros. El **Anexo 10** se presenta para información de los Miembros.

Los comentarios sobre los **Anexos 2** a **16** de este informe deben enviarse a la sede de la OIE antes del **10 de julio de 2020** para consideración en la reunión de septiembre de 2020 de la Comisión para los Animales Acuáticos. Los comentarios recibidos después de esta fecha no se presentarán para consideración de la Comisión.

Todos los comentarios deberán remitirse al Departamento de Normas: standards.dept@oie.int.

Los comentarios deberán presentarse en formato Word en lugar de PDF, puesto que los archivos PDF son difíciles de incorporar en los documentos de trabajo de la Comisión.

Los comentarios se deberán presentar en el anexo pertinente e incluir el nuevo texto propuesto, respaldado por una justificación estructurada o por referencias científicas publicadas. Las propuestas de supresión de texto deberán indicarse "~~con tachado~~" y las de modificación, "con doble subrayado". Los Miembros no deberán recurrir a la función automática de "control de cambios" del procesador de textos, ya que dichos cambios se pierden al compilar las propuestas de los Miembros en los documentos de trabajo de la Comisión. También se solicita a los Miembros que no reproduzcan el texto completo de un capítulo, puesto que así se pueden perder comentarios durante la preparación de los documentos de trabajo.

La Comisión para los Animales Acuáticos alienta encarecidamente a los Miembros a participar en el desarrollo de las normas internacionales de la OIE, presentando sus comentarios sobre este informe y preparando con anticipación su participación en el proceso de adopción en la Sesión General.

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1. BIENVENIDA DEL DIRECTOR GENERAL ADJUNTO

El Dr. Matthew Stone, director general adjunto de la OIE de Normas Internacionales y Ciencia, dio la bienvenida a la Comisión, agradeció a sus integrantes por sacar tiempo de su cargada agenda para apoyar el trabajo de la OIE y extendió las gracias a sus empleadores y gobiernos nacionales.

Además, detalló la participación de la OIE en el marco de la respuesta internacional frente a la pandemia de COVID-19, liderada por la Organización Mundial de la Salud.

El Dr. Stone señaló que el proyecto del 7.º Plan Estratégico se había distribuido recientemente a los Delegados y resumió las revisiones del organigrama de la sede de la OIE realizadas a finales de 2019 resultado de los procesos de evaluación vinculados con el desarrollo de la estrategia. Se refirió a las iniciativas en materia de aplicación de buenas prácticas reglamentarias, incluidas las expectativas relacionadas con la supervisión regulatoria, la finalización de la fase de diseño del Observatorio de la OIE y el inicio de un sistema de comentarios en línea para la elaboración y el examen de normas. Además, destacó la intención de alcanzar una articulación más clara del sistema científico de la OIE, a partir de la labor realizada en los últimos años y destinada a describir más claramente las expectativas de los centros de referencia en cuanto a la gestión de los procesos y el rendimiento; por otra parte, se comprometió a seguir colaborando con las comisiones especializadas durante esta labor.

Por último, presentó una actualización del sistema de gestión del desempeño de las comisiones, centrándose en la fase de evaluación que se iniciará en el segundo semestre de 2020, antes de las próximas elecciones para las comisiones especializadas en 2021.

2. APROBACIÓN DEL ORDEN DEL DÍA

El orden del día adoptado de la reunión figura en el Índice.

3. REUNIÓN CON LA DIRECTORA GENERAL

La Dra. Monique Eloit, directora general de la OIE, reconoció el trabajo considerable de la Comisión para los Animales Acuáticos y le agradeció su compromiso constante. Igualmente, dio las gracias a sus miembros y a la secretaría por su labor en la finalización de la Estrategia de la OIE para los animales acuáticos. Resaltó la importancia de la estrategia para la Organización que brindará una clara visión estratégica destinada a reforzar los esfuerzos del personal de la OIE, de los Miembros, socios y donantes encaminados a mejorar la sanidad y el bienestar de los animales acuáticos en todo el mundo.

4. COOPERACIÓN CON OTRAS COMISIONES ESPECIALIZADAS

La Comisión para los Animales Acuáticos se reunió con las secretarías de la Comisión de Normas Sanitarias para los Animales Terrestres (Comisión del Código) y de la Comisión de Normas Biológicas para compartir información y explorar ámbitos de interés común.

5. ESTRATEGIA DE LA OIE SOBRE LA SANIDAD DE LOS ANIMALES ACUÁTICOS

La Comisión de Animales Acuáticos prosiguió su labor para ultimar el proyecto de la Estrategia de la OIE para la sanidad de los animales acuáticos (Estrategia de la OIE para los animales acuáticos).

La directora general de la OIE se comprometió a desarrollar una estrategia para la sanidad de los animales acuáticos en la 4.ª Conferencia mundial de la OIE sobre la sanidad de los animales acuáticos, en Chile (abril de 2019), y reiteró este compromiso en la 87.ª Sesión General en mayo de 2019.

El desarrollo de la Estrategia de la OIE es una iniciativa liderada por la Organización con el apoyo de la Comisión para los Animales Acuáticos de la OIE, que reconoce la creciente importancia de la sanidad de los animales acuáticos y la necesidad de un enfoque estratégico para su gestión a escala mundial. Esta estrategia apoya el 7.º Plan Estratégico de la OIE, se inscribe plenamente dentro de su mandato y se ha elaborado en gran medida a partir de los aportes de la comunidad de la OIE para reflejar las principales necesidades comunes de los Miembros.

Se recabaron las opiniones de los Miembros y de los expertos de la OIE a través de diversos foros, entre ellos la Sesión General de la OIE, las conferencias de las comisiones regionales, las conferencias mundiales y los temas técnicos. A través de estos foros, se impuso la necesidad de una estrategia para los animales acuáticos y surgieron temas claros para definir sus objetivos.

Una vez identificados los objetivos de la estrategia, el siguiente paso fue recabar información de la comunidad de la OIE sobre las actividades prioritarias. En diciembre de 2019, la OIE llevó a cabo una consulta en la que participaron los Miembros (Delegados de la OIE y puntos focales de la OIE en el área), las oficinas regionales y subregionales, los centros de referencia y otros socios internacionales.

En la consulta, se solicitaron opiniones centradas en cuatro áreas: i) las actividades de la OIE que ya están funcionando bien; ii) las principales oportunidades; iii) las mayores amenazas para el crecimiento de la producción de animales acuáticos en los próximos 5-10 años, y iv) las prioridades de las actividades propuestas dentro de la estrategia.

Las respuestas representaron un valioso aporte a la hora de estructurar la estrategia, confirmaron las necesidades de los Miembros y priorizaron las actividades que debían incluirse.

Reunión de febrero de 2020

Durante su reunión de febrero de 2020, la Comisión para los Animales Acuáticos se reunió con algunas personas de la sede de la OIE para presentar el proyecto e invitarlas a formular observaciones, etapa que la Comisión consideró fundamental, ya que la estrategia constituye una actividad transversal cuya implementación implicará a numerosos departamentos de la OIE.

Tras la validación de la Dra. Eloit, se había previsto que la Estrategia de la OIE se pusiera en marcha en el marco de la 88.ª Sesión General de mayo de 2020. Se determinarán planes alternativos para su lanzamiento.

6. PLAN DE TRABAJO DE LA COMISIÓN DE NORMAS SANITARIAS PARA LOS ANIMALES ACUÁTICOS

Se recibieron comentarios de Chile, Nueva Caledonia y la UE.

La Comisión para los Animales Acuáticos analizó los comentarios de los Miembros y reiteró que trataría las mercancías seguras durante la revisión de la estructura del Artículo X.X.3 de todos los capítulos específicos de enfermedad como figura en su plan de trabajo, ya acordado en reuniones anteriores, en el ítem "mejora de las normas en las secciones 8-11". La Comisión acordó continuar con esta labor antes de febrero de 2021.

La Comisión examinó una solicitud para que se reconsiderara el orden de prioridad de las enfermedades restantes para la evaluación de las especies susceptibles; sin embargo, decidió no modificar el plan de trabajo, acordado en reuniones anteriores, puesto que ya se está implementado con los correspondientes grupos *ad hoc*.

7. *CÓDIGO SANITARIO PARA LOS ANIMALES ACUÁTICOS* DE LA OIE

La Comisión para los Animales Acuáticos indicó que, en este informe, no se brindaba ninguna justificación de los cambios de naturaleza editorial.

7.1. Textos propuestos para adopción en mayo de 2021

7.1.1. Nuevo proyecto de capítulo sobre bioseguridad para los establecimientos de acuicultura (Capítulo 4.X)

Se recibieron comentarios de Argentina, Australia, Canadá, Chile, Cuba, Estados Unidos de América, Japón, Nueva Caledonia, Nueva Zelanda, Nicaragua, Noruega, Taipéi Chino, la UE y AU-IBAR.

Contexto

El nuevo proyecto de capítulo sobre la bioseguridad para los establecimientos de acuicultura (Capítulo 4.X) es el segundo nuevo capítulo que formará parte de la revisión en curso del Título 4, *Prevención y control de las enfermedades*. El proyecto de capítulo se difundió tres veces para comentario entre septiembre de 2018 y septiembre de 2019.

En la reunión de septiembre de 2019, la Comisión para los Animales Acuáticos revisó el capítulo como respuesta a dos importantes temas sugeridos por los Miembros: vacío sanitario y bioseguridad a nivel del compartimento.

Informes de la Comisión donde se discutió del tema:

Informe de septiembre de 2018 (ítem 2.9, página 61); informe de febrero de 2019 (ítem 2.1, página 103); informe de septiembre de 2019 (ítem 6.1, páginas 21 y 33).

Reunión de febrero de 2020

Comentarios generales

La Comisión tomó nota de que las observaciones recibidas apoyaban el proyecto de capítulo y que la mayoría de los comentarios eran de carácter editorial.

La Comisión recordó a los Miembros que los términos del Glosario no aparecen en cursiva en los títulos.

Artículo 4.X.3 Introducción

Para más coherencia con otras partes del capítulo, la Comisión acordó añadir "bienestar" a la frase inicial del primer párrafo.

La Comisión acordó añadir "pueden" en el último párrafo, "Entre los beneficios pueden...", señalando que la implementación de la bioseguridad no se traduciría necesariamente en un mejor acceso al mercado.

Artículo 4.X.4 Principios generales

La Comisión no aceptó enmendar la definición de "bioseguridad" del Glosario, en consonancia con el *Código Terrestre*. La Comisión observó que los términos "infección", "infestación" y "enfermedad" se utilizan de manera diferente en ambos *Códigos*.

En el párrafo 1, la Comisión acordó trasladar "planificación" al principio de la segunda frase del primer párrafo para subrayar que este componente representa un primer paso importante para la bioseguridad.

En el párrafo 1, la Comisión no estuvo de acuerdo con suprimir "en un establecimiento de acuicultura" porque el ámbito de aplicación de este capítulo es específico de la bioseguridad en los establecimientos de acuicultura.

La Comisión agregó un nuevo apartado 5) para abordar la cuestión de la señalización, ya que consideró que se trataba de una herramienta importante destinada a aumentar la sensibilización y la comprensión de determinadas medidas de bioseguridad.

En el apartado 1, en relación con las posibles rutas de transmisión, la Comisión suprimió la referencia al Artículo 4.X.5, puesto que esta referencia cruzada no era correcta, ya que se refiere a categorías de sistemas de producción acuícola y no a posibles rutas de transmisión.

La Comisión estuvo de acuerdo con un comentario que destacaba la importancia de que a los visitantes de los establecimientos acuícolas se les informara sobre el plan de seguridad y sus exigencias con miras a asegurar su cumplimiento. Sin embargo, la Comisión consideró que la mejor manera de abordar esta cuestión era añadiendo un nuevo apartado 7, *Personal y visitantes*, en el Artículo 4.X.6, *Rutas de transmisión y medidas de mitigación*.

La Comisión aclaró que se pueden realizar auditorías externas a solicitud de clientes o entidades reguladoras, como se señala en el apartado 7.

Artículo 4.X.5 Categorías de los sistemas de producción acuícola

La Comisión acordó añadir "sistema" a todos los títulos de las categorías.

La Comisión no aceptó añadir "dispensadores de alimentos" como ejemplo de sistemas semi-cerrados por no ser un término de uso común. Señaló también que las jaulas flotantes se consideraban sistemas semi-cerrados porque existe un control parcial sobre el agua que entra y sale del sistema.

En el caso de los sistemas cerrados, y en aras de claridad, la Comisión acordó desplazar la última frase, "Las condiciones ambientales también se pueden controlar", al principio del párrafo.

Artículo 4.X.5 bis Manejo por áreas

La Comisión no estuvo de acuerdo con incluir una definición en el Glosario de "manejo por áreas", ya que consideró que el contexto de este término era claro y que se utilizaba con poca frecuencia en todo el *Código Acuático*.

La Comisión no acordó eliminar el término "áreas" en todo el texto de este artículo y sustituirlo por "zonas", señalando que los establecimientos de acuicultura pueden situarse en áreas geográficas definidas (por ejemplo, bahías) en lugar de "zonas" con un estatus sanitario específico.

La Comisión aceptó añadir "la coordinación de" en la última frase del artículo para destacar que se requiere cooperación a la hora de aplicar medidas de bioseguridad entre los establecimientos de acuicultura en cuerpos de aguas compartidas.

La Comisión analizó la pertinencia de destacar la importancia de la comunicación permanente entre los establecimientos acuícolas, pero convino en que no era necesaria, ya que la "coordinación" entre los establecimientos acuícolas incluye e implica dicha comunicación.

Artículo 4.X.6 Rutas de transmisión y medidas de mitigación

1. Animales acuáticos

En cuanto al apartado 1, relativo a los riesgos de transmisión asociados a los animales acuáticos, la Comisión no estuvo de acuerdo con elaborar requisitos para los países exportadores. Consideró que esta cuestión quedaba fuera del ámbito de aplicación del capítulo, que se limita a la bioseguridad de los establecimientos de acuicultura. La Comisión convino en que la redacción del apartado 1(b) no era clara, que podía contradecir lo indicado en el apartado 1(a) y la modificó para establecer que, si se introducen animales acuáticos con estatus desconocido, los mismos deben ponerse en cuarentena.

La Comisión no estuvo de acuerdo en que las condiciones de la cuarentena debían describirse como "propicias para la expresión clínica". Estimó que la definición del Glosario de “cuarentena” resultaba suficiente para los fines del presente capítulo. Sin embargo, reconoció que podía ser beneficioso contar con mayores recomendaciones sobre la cuarentena y acordó examinar esta cuestión en el contexto de su plan de trabajo.

En el apartado 1(d), la Comisión no estuvo de acuerdo con la propuesta de incluir el "estrés" señalando que el tema central de este apartado es la exposición a los agentes patógenos.

En cuanto al apartado 1(e), la Comisión convino en subrayar que los desplazamientos de animales acuáticos entre distintas poblaciones sólo debían realizarse con miras a mantener el estatus sanitario más alto posible de las poblaciones y modificó el texto en consecuencia.

En el apartado 1(h), si bien la Comisión reconoció la certeza de un comentario que sugería que las autoridades competentes debían seguir las directrices de la OIE relativas a la notificación de sospechas de enfermedad, no aceptó suprimir en el texto la referencia a los requisitos locales. La Comisión explicó que los requisitos locales constituyen la base legal para la notificación a la autoridad competente y pueden exceder las obligaciones de notificación de la autoridad competente a la OIE. La Comisión no aceptó añadir "por los Servicios de sanidad de los animales acuáticos bajo la dirección de la autoridad competente" al final de este apartado, señalando que tales investigaciones y el diagnóstico de la causa de la mortalidad pueden realizarse a nivel de la granja en algunas circunstancias.

En la última frase del apartado 1(i), la Comisión rechazó la propuesta de declarar que el vacío sanitario debía coordinarse entre los establecimientos de acuicultura sujetos a un acuerdo de manejo por áreas, ya que consideró que era posible una coordinación sincrónica del vacío sanitario en los establecimientos de acuicultura en aguas compartidas sin un acuerdo oficial.

En cuanto al apartado 1(i), la Comisión no estuvo de acuerdo con sustituir "infección" por "agentes patógenos" porque el tema de este apartado son los ciclos de infección.

La Comisión no aceptó añadir un nuevo apartado 1(k) con vistas a abarcar los registros de traslado debido a que el mantenimiento de los mismos no constituye una medida de mitigación. Igualmente, señaló que el mantenimiento de registros se contempla en el apartado 2(b) del Artículo 4.X.8, *Desarrollo del plan de bioseguridad*.

2. Productos y residuos de animales acuáticos

En el apartado 2, relativo a los riesgos de transmisión asociados a los productos y residuos de animales acuáticos, la Comisión acordó suprimir "animales acuáticos" en la última frase del primer párrafo para mejorar la legibilidad.

En el segundo párrafo, la Comisión aceptó suprimir la penúltima frase relativa a los residuos de alto riesgo, por considerarlo una repetición.

En el apartado 2(b), la Comisión no acordó añadir "y la adopción de medidas de bioseguridad" porque su adopción está implícita en la implementación de un plan de bioseguridad.

En el apartado (c), sustituyó "sistemas" por "procedimientos" en la primera frase y armonizó la última frase con la redacción utilizada en el anterior apartado (b). No acordó añadir "eliminación" porque la "colecta" incluye la eliminación, ya sea la eliminación de las jaulas o del establecimiento de acuicultura.

3. Agua

En cuanto al apartado 3, relativo a los riesgos de transmisión asociados con el agua, la Comisión no acordó modificar la frase "un riesgo de introducción en, de propagación dentro y de liberación de agentes patógenos desde los establecimientos de acuicultura" en el primer párrafo, dado que esta terminología se utiliza en todo el capítulo.

La Comisión no aceptó suprimir la última frase del apartado 3(b): "El nivel de tratamiento requerido dependerá de los riesgos identificados", dado el importante contexto que brinda. Añadió "tipo y el" a la frase para subrayar que la selección del método de tratamiento dependerá de los riesgos identificados.

La Comisión acordó añadir "y de los residuos filtrados asociados" en el apartado 3(c) porque este tipo de desechos no se especifica en ninguna otra parte del capítulo, así como agregar "tipo" a la última frase porque la selección de un método o métodos de desinfección se determinará por su eficacia contra el agente patógeno específico. Por otra parte, suprimió la palabra "contención", por considerarla superflua.

En respuesta a los comentarios, se añadió un nuevo apartado 3(f) para abordar las medidas destinadas a mitigar el riesgo de que el agua entre en el establecimiento de acuicultura a través del traslado de animales acuáticos. La Comisión destacó que en el Capítulo 5.5, *Control de riesgos para la sanidad de los animales acuáticos asociados al transporte de estos animales*, se proporcionan más orientaciones sobre la gestión del agua que se utiliza en el transporte.

4. Alimentos para animales (piensos)

La Comisión no estuvo de acuerdo con añadir un nuevo apartado para subrayar la importancia de la gestión y el almacenamiento adecuados de los piensos *in situ* y de las prácticas para mitigar el riesgo de contaminación de los mismos, ya que consideró que esto se reflejaba con suficiente detalle en el texto propuesto.

5. Fómites

En el apartado 5(a), relativo a los riesgos de transmisión asociados a los fómites, la Comisión acordó enmendar el texto para incluir los fómites trasladados dentro y desde el establecimiento de acuicultura.

La Comisión no acordó especificar que los riesgos de enfermedad transmitidos por los fómites que estuvieron en contacto directo con animales acuáticos debían ser objeto de una atención particular porque la evaluación del riesgo aborda todos los niveles de riesgo de enfermedad.

6. Vectores

En el apartado 6, relativo a los riesgos de transmisión asociados a los vectores, la Comisión acordó sustituir "riesgo" por "probabilidad" en el segundo párrafo. Se realizaron modificaciones similares en todo el capítulo, cuando fuera pertinente, en aras de reflejar claramente lo expresado.

En el apartado 6(a), la Comisión efectuó un cambio editorial a efectos de legibilidad y no acordó añadir "o desinfección" en el punto a(i) aduciendo que cubre únicamente las medidas de mitigación física.

En el apartado 6(b), la Comisión no aceptó añadir una frase indicando que la lucha contra las plagas debía llevarse a cabo de manera ética y respetuosa del medio ambiente porque esta perspectiva no entra en el ámbito de aplicación del capítulo.

7. Personal y visitantes

En respuesta a un comentario, la Comisión añadió un nuevo apartado 7, *Personal y visitantes*, en el que se recomienda que se informe a los visitantes de los establecimientos acuícolas sobre el plan de bioseguridad, con el fin de garantizar su cumplimiento.

La Comisión convino en que la señalización era importante para aumentar en términos de sensibilización y llegó a la conclusión de que se inscribiría mejor en el Artículo 4.X.4, *Principios generales*, y no en el Artículo 4.X.6, *Rutas de transmisión y medidas de mitigación*.

Artículo 4.X.7 Análisis de riesgo

La Comisión tomó nota de la solicitud de incluir el potencial establecimiento de agentes patógenos en el medio ambiente en los descriptores cualitativos de las consecuencias en la Tabla 2. Sin embargo, se negó a hacer el cambio sugerido porque el capítulo se refiere a la bioseguridad de los establecimientos de acuicultura y, por consiguiente, la evaluación del riesgo para el establecimiento de agentes patógenos en el medio ambiente queda fuera de su ámbito de aplicación.

La Comisión rechazó la supresión de la referencia al comercio en la Tabla 2, señalando que, a efectos del presente capítulo, el comercio descrito a nivel del establecimiento de acuicultura es pertinente.

En la Tabla 3, la Comisión no aceptó cambiar el título por "Matriz de evaluación del riesgo" al considerar que era claro y exacto tal como estaba escrito. Sin embargo, sustituyó "medio" por "bajo" en la combinación entre consecuencia insignificante y posibilidad segura, ya que acordó que se trataba de una estimación del riesgo más apropiada.

En la Etapa 3, la Comisión no aceptó eliminar "eficaces" del primer párrafo porque consideró que era una parte importante de la priorización de las medidas de mitigación.

Artículo 4.X.8 Desarrollo del plan de bioseguridad

En el apartado 1(a) relativo a la elaboración de un plan de bioseguridad, la Comisión acordó añadir "campo de aplicación" por ser parte importante del plan de bioseguridad. También reconoció que el plan debía incluir información sobre los puntos de acceso al establecimiento de acuicultura y añadió un texto para incorporar esta cuestión en el apartado 1(b).

En el apartado 2, la Comisión agregó "medidas de cuarentena" en los ejemplos de la documentación requerida en 2(b). La Comisión no aceptó incluir "requisitos reglamentarios" por tratarse en otra parte del capítulo.

En el apartado 2(c), rechazó la propuesta de especificar que los requisitos de notificación debían limitarse a la comunicación con las autoridades competentes porque los procedimientos podían incluir la notificación a la dirección del establecimiento de acuicultura o a profesionales privados de la sanidad de los animales acuáticos.

En el apartado 2(c), no aceptó añadir "planes de contingencia" al título porque los planes de contingencia están fuera del ámbito de aplicación de este capítulo.

El nuevo Capítulo 4.X. revisado, *Bioseguridad para los establecimientos de acuicultura*, figura en el Anexo 2 para comentario de los Miembros.

La aprobación del capítulo se ha pospuesto hasta la 89.ª Sesión General de mayo de 2021. Sabiendo que el capítulo ya ha sido objeto de amplias consultas, se pide a los Miembros que sólo presenten observaciones para abordar cuestiones de fondo que no hayan sido examinadas anteriormente.

7.1.2. Inclusión en la lista de la OIE de la infección por el virus iridiscente de los decápodos tipo 1 (DIV1) – Artículo 1.3.3 revisado del Capítulo 1.3

Se recibieron comentarios de Nicaragua, Nueva Caledonia, Taipéi Chino y la UE.

Contexto

La Comisión, en su reunión de febrero de 2019, evaluó si la infección por el virus iridiscente del hemocito del camarón cumplía con los criterios de inclusión en la lista de las enfermedades de los animales acuáticos descritos en el Artículo 1.2.2 y acordó que la infección cumple con los criterios de inclusión de la lista de la OIE para añadirla en el Artículo 1.3.3 relativo a las enfermedades de los crustáceos que forman parte de la lista de la OIE. La Comisión aceptó que el nombre de la enfermedad debía cambiarse por "Infección por el virus iridiscente de los decápodos tipo 1", de acuerdo con la clasificación del agente patógeno en la base de datos del Comité Internacional para la Taxonomía de los Virus (ICTV, por sus siglas en inglés).

La propuesta de incluir la infección por DIV1 en la lista se difundió tres veces para comentario y, en cada ocasión, se incluyó, a título informativo, la evaluación con respecto a los criterios de inclusión en la lista.

Informes de la Comisión donde se discutió este tema:

Informe de febrero de 2019 (ítem 3.1.1, página 113); informe de septiembre de 2019 (ítem 6.2, página 45).

Reunión de febrero de 2020

La Comisión observó que todas las observaciones recibidas apoyaban la inclusión de la infección por DIV1.

En respuesta a una solicitud que sugería la introducción de acrónimos en la lista de enfermedades, la Comisión recordó que no era una convención que se aplicara al Capítulo 1.3 y que los acrónimos y los nombres comunes de las enfermedades se utilizan en los capítulos específicos de enfermedad.

El Artículo 1.3.3 revisado del Capítulo 1.3, *Enfermedades de la lista de la OIE*, figura en el Anexo 3 para cometario de los Miembros (también ver ítem 7.3.4.).

La aprobación del capítulo se ha pospuesto hasta la 89.ª Sesión General de mayo de 2021. Sabiendo que el artículo ya ha sido objeto de amplias consultas, se pide a los Miembros que sólo presenten observaciones para abordar cuestiones de fondo que no hayan sido examinadas anteriormente.

7.1.3. Modelo de Artículo 10.X.13 para los capítulos 10.5, 10.6 y 10.10 específicos de enfermedades de los peces (y Artículo 10.4.17 del Capítulo 10.4, Infección por el virus de la anemia infecciosa del salmón)

Se recibieron comentarios de Australia, Chile, Taipéi Chino y la UE.

Contexto

La revisión del Artículo 10.X.13, *Importación, para la acuicultura, de huevos desinfectados de un país, una zona o un compartimento no declarados libres de infección por X*, para los capítulos 10.5, 10.6 y 10.10 específicos de enfermedades de los peces (y el Artículo 10.4.17 del Capítulo 10.4), fue iniciada por la Comisión para los Animales Acuáticos en febrero de 2019 en respuesta a las solicitudes de aclaración del objetivo del artículo. El modelo de artículo se difundió dos veces para comentario.

Informes de la Comisión donde se discutió este tema:

Informe de septiembre de 2019 (ítem 6.3, página 47), reunión de febrero de 2020.

La Comisión tomó nota de que los comentarios recibidos apoyaban las enmiendas propuestas e incluían algunas modificaciones de carácter editorial.

La Comisión acordó incorporar en el primer párrafo una referencia cruzada al Capítulo 4.4, *Recomendaciones para la desinfección de la superficie de los huevos de salmónidos*, a fin de orientar a los Miembros sobre los protocolos de desinfección.

En cuanto al apartado 1, la Comisión no acordó incluir una recomendación para que las autoridades competentes de los países importadores obtuvieran garantías de la autoridad competente del país exportador por considerarlo implícito en el texto actual.

La Comisión no aceptó referirse al protocolo en el apartado 1(a) del Artículo 4.4.2 (del Capítulo 4.4), ya que otros protocolos pueden ser igualmente eficaces. Rechazó también un texto haciendo referencia al Artículo 4.4.1 (del Capítulo 4.4) en el apartado 1(c), ya que lo consideró un detalle innecesario y que la referencia al Capítulo 4.4 en el texto principal del apartado (1) era suficiente.

La Comisión modificó la primera frase del apartado 2 para aclarar que la autoridad competente del país importador no puede aplicar por sí misma medidas previas a la importación, como se deja claro en el Artículo 3.

En cuanto al apartado 2, la Comisión no aceptó incluir procedimientos para especies distintas de los salmónidos. Reiteró que, en varias ocasiones, había solicitado a los Miembros que proporcionaran protocolos para otras especies, pero sin respuesta hasta la fecha.

La Comisión rechazó la propuesta de eliminar "entre ellas" en el apartado 2. Señaló que su uso permite medidas adicionales a las enumeradas y que su supresión limitaría las opciones. La Comisión decidió que la desinfección a la llegada no debía constituir un requisito, sino una medida posterior a la importación que un Miembro debía tomar en consideración.

La Comisión modificó el apartado 3 para limitar la aplicación del certificado sanitario internacional a los apartados 2 (a) y (b), porque las medidas internas que también figuran en el apartado 2 no son relevantes para el certificado internacional.

El modelo de Artículo 10.X.13 revisado para los capítulos 10.5, 10.6 y 10.10 (y el Artículo 10.4.17 para el Capítulo 10.4) figura en el Anexo 4 y se presenta para comentario de los Miembros.

La aprobación del artículo se ha pospuesto hasta la 89.ª Sesión General de mayo de 2021. Sabiendo que el artículo ya ha sido objeto de amplias consultas, se pide a los Miembros que sólo presenten observaciones para abordar cuestiones de fondo que no hayan sido examinadas anteriormente.

7.1.4. Artículo 10.9.2 del Capítulo 10.9 Infección por el virus de la viremia primaveral de la carpa

Se recibieron comentarios de Canadá, China (Rep. Pop.), Estados Unidos de América y Taipéi Chino.

Contexto

La lista revisada de especies susceptibles del Artículo 10.9.2 del Capítulo 10.9, *Infección por el virus de la viremia primaveral de la carpa*, fue adoptada en la 87.ª Sesión General en mayo de 2019. Sin embargo, a la luz de las nuevas pruebas científicas sobre la susceptibilidad del pez cebra a la infección por el virus de la viremia primaveral de la carpa, la Comisión para los Animales Acuáticos revisó la evaluación anterior de esta especie y acordó que el pez cebra cumplía los criterios de inclusión en la lista de especies susceptibles y que, por lo tanto, debía incluirse en el Artículo 10.9.2. Esta propuesta se distribuyó para comentario en el informe de la Comisión de septiembre de 2019.

Reunión de febrero de 2020

La Comisión para los Animales Acuáticos señaló que los Miembros apoyaban la inclusión del pez cebra *(Danio rerio)* en la lista de especies susceptibles del Artículo 10.9.2.

Se solicitó la inclusión en el Artículo 10.9.2 de la perca amarilla *(Perca flavescens)* y del salmón rojo *(Oncorhynchus nerka)* como especies susceptibles a la infección por el virus de la viremia primaveral de la carpa, en base a las pruebas del estudio de Emmenegger *et al.*, 2016. En su respuesta, la Comisión recordó a los Miembros que las evaluaciones de estas dos especies realizadas por el Grupo *ad hoc* sobre la susceptibilidad de las especies de peces a las enfermedades de la lista de la OIE, en noviembre de 2017, habían examinado dicho documento. El grupo *ad hoc* había llegado a la conclusión de que esas especies no cumplían con los criterios de susceptibilidad porque la vía de infección experimental descrita en este estudio era invasiva. La Comisión reiteró su decisión anterior de no incluir estas dos especies como susceptibles.

La Comisión informó a los Miembros de que, si deseaban proponer la inclusión de especies adicionales, debían remitirse a los informes pertinentes de los grupos *ad hoc* (https://www.oie.int/es/normas/comisiones-especializadas-y-grupos-de-trabajo-y-ad-hoc/grupos-ad-hoc-informes/), y presentar nuevas pruebas científicas en apoyo de su propuesta.

Referencias: Emmenegger, A. J., Sanders, G. E., Conway, C. M., Binkowski, F. P., Winton, J. R. and Kurath, G. (2016). Experimental infection of six North American fish species with the North Carolina strain of spring Viremia of Carp Virus. Aquaculture, 450, 273–282.

El Artículo 10.9.2 revisado del Capítulo 10.9, *Infección por el virus de la viremia primaveral de la carpa*, figura en el Anexo 5 para comentario de los Miembros. La aprobación del artículo se ha pospuesto hasta la 89.ª Sesión General de mayo de 2021. Sabiendo que el artículo ya ha sido objeto de amplias consultas, se solicita a los Miembros que sólo presenten observaciones para abordar cuestiones de fondo que no hayan sido examinadas anteriormente.

7.1.5. Artículo 10.10.2 del Capítulo 10.10 Infección por el virus de la septicemia hemorrágica viral

Se recibieron comentarios de Canadá, Corea (Rep. de), Estados Unidos de América, Tailandia y la UE.

Contexto

El Grupo *ad hoc* sobre la susceptibilidad de las especies de peces a la infección por las enfermedades de la lista de la OIE aplicó los criterios de inclusión en la lista de especies susceptibles a la infección por el virus de la septicemia hemorrágica viral, de conformidad con el Capítulo 1.5, *Criterios para la inclusión de especies susceptibles de infección por un agente patógeno específico* (informe disponible en [https://www.oie.int/es/normas/comisiones-especializadas-y-grupos-de-trabajo-y-ad-hoc/grupos-ad-hoc-informes/]. Las evaluaciones fueron revisadas por la Comisión en su reunión de septiembre de 2019 y la lista modificada de especies susceptibles del Artículo 10.10.2 se distribuyó para comentario por primera vez en el informe de la Comisión de septiembre de 2019.

Informes de la Comisión donde se discutió este tema:

Informe de septiembre de 2019 (ítem 6.4, página 49).

Reunión de febrero de 2020

La Comisión confirmó que el pez cebra *(Danio rerio)* cumple los criterios de inclusión en la lista de especies susceptibles y, aunque Cho *et al.* (2019) demostraron la infección por una ruta natural de transmisión (inmersión), este estudio fue dejado de lado por inadvertencia en el informe del grupo *ad hoc*. Las especies pueden cumplir los criterios de inclusión a partir de estudios experimentales, pero, en esos casos, es necesario considerar si los procedimientos experimentales (por ejemplo, inyección o carga infecciosa) imitan las rutas naturales de transmisión de la enfermedad.

En respuesta a un comentario sobre la interpretación de la aplicación de los criterios de inclusión en la lista de especies susceptibles, la Comisión reconoció que, si bien no había cambiado la interpretación de las pruebas necesarias para evaluar una especie como susceptible, convino en que no se había descrito suficientemente en los informes anteriores. La Comisión subrayó que, como se señalaba en el informe de septiembre de 2019 del grupo *ad hoc*, si un solo estudio proporcionaba evidencias de la susceptibilidad de una especie, también se requería algún tipo de prueba corroborativa, para satisfacer los criterios de susceptibilidad. Las pruebas de corroboración pueden encontrarse en el documento de referencia científica o en otros documentos.

Varios Miembros formularon observaciones sobre la inclusión de genotipos del virus de la septicemia hemorrágica viral en el cuadro de especies susceptibles del Artículo 10.10.2. La Comisión aceptó suprimir los genotipos del cuadro debido a que el conocimiento de la susceptibilidad de las especies por genotipo era incompleto y la información no era pertinente a efectos del comercio. Cualquier diferenciación de las medidas comerciales basadas en el genotipo requeriría que la Comisión evaluara el caso de la diferenciación de la cepa del virus de la septicemia hemorrágica viral utilizando el enfoque que la Comisión aplicase anteriormente al virus de la anemia infecciosa del salmón.

Sin embargo, la Comisión convino en que la información sobre los genotipos del virus de la septicemia hemorrágica viral debía incluirse en el capítulo consagrado a esta enfermedad en el *Manual Acuático* en la lista de especies susceptibles (2.2.1) y en la lista de especies con pruebas incompletas de susceptibilidad (2.2.2), ya que se trata de una valiosa información epidemiológica. La Comisión tomó nota de que el Capítulo 2.3.10, *Septicemia hemorrágica viral*, del *Manual Acuático* se está estructurado a partir del nuevo modelo y que la lista de genotipos será revisada por la secretaría de la OIE de acuerdo con esta propuesta.

La Comisión decidió establecer claramente cuando existe una falta de información sobre el genotipo de una especie susceptible para evitar toda confusión.

La Comisión pidió a la secretaría de la OIE que revisara la lista de referencias del informe del grupo *ad hoc* para corregir los errores, introducir las modificaciones necesarias y actualizar la versión en el sitio web de la OIE.

Un Miembro solicitó que se considerara la posibilidad de incluir la trucha de arroyo *(Salvelinus fontinalis)* y la lota *(Lota lota)* en la lista de especies susceptibles al virus de la septicemia hemorrágica viral sobre la base de información no publicada. La Comisión reiteró que i) el grupo *ad hoc* sólo puede examinar la información publicada y ii) estas dos especies habían sido evaluadas por el grupo *ad hoc* y se había determinado que no cumplían los criterios de susceptibilidad. La Comisión alentó a los Miembros a que proporcionaran toda nueva prueba científica para su revisión.

La Comisión no estuvo de acuerdo con añadir "platija olivácea" como nombre común del falso halibut del Japón *(Paralichthys olivaceus)* y recordó a los Miembros que se había convenido hacer referencia a los nombres comunes de las especies de peces en consonancia con FAOTERM (http://www.fao.org/faoterm/es/) y a los nombres científicos de los peces en consonancia con la base terminológica Fishbase (https://www.fishbase.se/search.php).

La Comisión modificó el nombre científico del bagre pardo de "*Ictalurus nebulosus*" por "*Ameiurus nebulosus*", en consonancia con la denominación que figura en Fishbase.

Referencia: Cho, S. Y., Protzman, R. A., Kim, Y. O., Vaidya, B., Oh, M. J., Kwon, J. and Kim, D. (2019). Elucidation of mechanism for host response to VHSV infection at varying temperatures *in vitro* and *in vivo* through proteomic analysis. Fish and Shellfish Immunology, 88, 244–253.

El Artículo 10.10.2 revisado del Capítulo 10.10, *Infección por el virus de la septicemia hemorrágica viral*, figura en el Anexo 8 para comentario de los Miembros.

La aprobación del artículo se ha pospuesto hasta la 89.ª Sesión General de mayo de 2021. Sabiendo que el artículo ya ha sido objeto de amplias consultas, se pide a los Miembros que sólo presenten observaciones para abordar cuestiones de fondo que no hayan sido examinadas anteriormente.

7.1.6. Definiciones del Glosario de "residuos de animales acuáticos" y "productos de animales acuáticos"

Se recibieron comentarios de Chile, Canadá, Nueva Zelandia, la UE y AU-IBAR.

Contexto

En su reunión de septiembre de 2019, la Comisión para los Animales Acuáticos propuso una nueva definición del Glosario para "residuos de animales acuáticos", dado que el término se utiliza ampliamente en el nuevo proyecto de capítulo sobre *Bioseguridad para los establecimientos de acuicultura* (Capítulo 4.X), así como en el Capítulo 4.7, *Manipulación, eliminación y tratamiento de residuos de animales acuáticos*. La nueva definición del Glosario se difundió para comentario en el informe de la Comisión de septiembre de 2019.

Informes de la Comisión donde se discutió este tema:

Informe de septiembre de 2019 (ítem 6.7, página 79).

Reunión de febrero de 2020

La Comisión examinó los comentarios y modificó la definición de "residuos de animales acuáticos" para hacer la distinción entre los productos de animales acuáticos y los residuos correspondientes y para distinguir los residuos de animales acuáticos de los subproductos de animales acuáticos. Como consecuencia de estas modificaciones, también introdujo cambios en la definición de "productos de animales acuáticos" para ajustarla a la nueva definición de "residuos de animales acuáticos".

La Comisión tomó nota de que, por convención, una vez que se adopte la nueva definición del Glosario de "residuos de animales acuáticos", se suprimirá la definición de "residuos de animales acuáticos" del Artículo 4.7.3.

Asimismo, la Comisión examinó el uso del término "residuos" en todo el *Código Acuático* y propuso que, una vez adoptada la nueva definición de "residuos de animales acuáticos", el término "residuos" se cambie por el término en cursiva "residuos de animales acuáticos", cuando sea pertinente, para reflejar el término definido.

Las definiciones revisadas del Glosario para “residuos de animales acuáticos” y “productos de animales acuáticos” figuran en el Anexo 7 para comentario de los Miembros.

La aprobación de las definiciones del Glosario se ha pospuesto hasta la 89.ª Sesión General de mayo de 2021. Sabiendo que ya ha sido objeto de amplias consultas, se pide a los Miembros que sólo presenten observaciones para abordar cuestiones de fondo que no hayan sido examinadas anteriormente.

7.2. Textos para comentario de los Países Miembros

7.2.1. Definición revisada del Glosario del término “vector”

En respuesta a una solicitud del Grupo *ad hoc* sobre la susceptibilidad de las especies de moluscos a la infección por las enfermedades de la lista de la OIE, la Comisión enmendó la definición de “vector” del Glosario para aclarar que los vectores de un agente infeccioso específico no pueden considerarse una especie susceptible para el mismo agente.

La definición revisada del Glosario de “vector” figura en el Anexo 7 para comentario de los Miembros.

7.2.2. Enfoques para determinar los periodos requeridos para demostrar la ausencia de enfermedad

Contexto

En el informe de la Comisión de septiembre de 2018, se distribuyó por primera vez para comentario un documento de debate sobre los enfoques para determinar los períodos requeridos con el fin de demostrar la ausencia de enfermedad. La Comisión examinó las observaciones recibidas y distribuyó un documento de debate revisado en su informe de septiembre de 2019.

Informes de la Comisión donde se discutió este tema:

Informe de septiembre de 2018 (ítem 2.10, página 71); informe de septiembre de 2019 (ítem 6.6, página 55).

Reunión de febrero de 2020

Se recibieron comentarios de Australia, Canadá, Chile, China (Rep. Pop.), Estados Unidos de América, Japón, Nicaragua, Noruega, Nueva Zelanda, Tailandia y la UE.

La Comisión tomó nota de las observaciones constructivas presentadas sobre el documento de debate y llegó a la conclusión de que el documento había cumplido su propósito. Basándose en los comentarios, la Comisión se centró en la revisión de los artículos modelo X.X.4, X.X.5 y X.X.6 de los capítulos específicos de enfermedad del *Código Acuático* sobre la ausencia de enfermedad y en el Capítulo 1.4.

Se redactaron modelos para los capítulos específicos de enfermedad, con el fin de abarcar cuestiones relacionadas con los procedimientos destinados a establecer la ausencia de enfermedad a nivel del país, la zona y el compartimento, por ejemplo, la vacunación y las condiciones básicas de bioseguridad, a las que se hará referencia en los artículos pertinentes del Capítulo 1.4.

Se mantendrá la estructura de los actuales artículos modelo X.X.4 a X.X.6 pero se proponen ciertas revisiones. La ausencia de especies susceptibles no constituirá un procedimiento de ausencia de enfermedad para los patógenos con un amplio rango de hospedadores (actualmente, este procedimiento no está disponible para la infección por *Aphanomyces invadans* – síndrome ulcerante epizoótico – y la infección por el virus de la septicemia hemorrágica viral). Se redactaron artículos separados orientados a establecer la ausencia de enfermedad para una zona y un compartimento y se incluyó un nuevo artículo sobre la restitución de la ausencia de enfermedad a nivel de compartimento. La Comisión decidió que siempre se requiere la vigilancia específica con el fin de establecer la ausencia de enfermedad de un compartimento (la ausencia histórica y la ausencia de especies susceptibles no son procedimientos adecuados para la ausencia de enfermedad a nivel de compartimento).

El Capítulo 1.4 será revisado para centrarse en las disposiciones de acompañamiento de los capítulos específicos de enfermedad del *Código Acuático*. El actual Artículo 1.4.6 se ampliará en varios artículos e incluirá los criterios y enfoques establecidos en el documento de debate (por ejemplo, las orientaciones sobre la vigilancia para alcanzar la ausencia de enfermedad). La información genérica sobre la vigilancia y los ejemplos de sistemas de vigilancia que figuran en el actual Capítulo 1.4 se acortarán considerablemente o se suprimirán.

El modelo de los Artículos X.X.4, X.X.5 y X.X.6 para los capítulos específicos de enfermedad del *Código Acuático* figuran en el Anexo 9 para comentario de los Miembros.

7.3. Textos para información de los Países Miembros

7.3.1. Retiro de la lista de la infección por el virus de la necrosis hipodérmica y hematopoyética infecciosa – Artículo 1.3.3 revisado del Capítulo 1.3

La Comisión recibió una solicitud de un Miembro para suprimir la infección por el virus de la necrosis hipodérmica y hematopoyética infecciosa de la lista de enfermedades del Artículo 1.3.3 del Capítulo 1.3, *Enfermedades de la lista de la OIE*. La Comisión aceptó seguir evaluando la cuestión en su reunión de septiembre de 2020 y solicitó a los Miembros que proporcionaran toda la información disponible relacionada con los criterios de inclusión en la lista de enfermedades de los animales acuáticos, concretamente, los criterios 4(b) y 4(c) del Artículo 1.2.2 (consecuencias para los animales acuáticos de cultivo o silvestres, respectivamente).

La Comisión informa a los Miembros que toda recomendación para la eliminación de la lista del virus de la necrosis hipodérmica y hematopoyética infecciosa se comunicará para su consideración, junto con una evaluación con respecto a los criterios de inclusión en la lista.

7.3.2. Nuevo proyecto de capítulo sobre la preparación para las respuestas de emergencia y la gestión de un brote de enfermedad

La Comisión aceptó trabajar en dos nuevos capítulos para el Título 4 revisado, *Prevención y control de las enfermedades*: i) Preparación para las respuestas de emergencia, y ii) Gestión de los brotes de enfermedades. La Comisión acordó definir la estructura de los artículos de ambos capítulos, dada la estrecha relación que existe entre ambos, y volver a examinar esta labor en su reunión de septiembre de 2020.

7.3.3. Infección por el virus del edema de la carpa

La Comisión examinó la información científica referente a la infección por el virus del edema de la carpa, debido a las recientes notificaciones en varios países de la región de Asia y el Pacífico y a la aparente progresión de su distribución geográfica. La Comisión observó que la enfermedad fue incluida en la lista de enfermedades de declaración obligatoria por la NACA (red de centros de acuicultura para la región Asia-Pacífico) en 2017; y hasta ahora ha afectado considerablemente la producción de carpas, que constituye la mayor producción pesquera del mundo.

A partir de la información científica disponible, la Comisión acordó que la infección por el virus del edema de la carpa cumple con la definición de la OIE de "enfermedad emergente" y, como tal, los Miembros deben notificarla de conformidad con el Artículo 1.1.4 del *Código Acuático*. La Comisión también alentó a los Miembros a investigar los eventos de mortalidad y morbilidad relacionados con esta enfermedad, haciendo hincapié en que un mejor conocimiento del virus es esencial para los esfuerzos de control de su posible propagación. Se insta a los Miembros a presentar a la Comisión información sobre sus experiencias con la infección por el virus del edema de la carpa y el impacto de la enfermedad para consideración en su reunión de septiembre de 2020.

7.3.4. Evaluación de la infección por el virus iridiscente de los decápodos tipo 1 para inclusión en el Capítulo 1.3 del *Código Sanitario para los Animales Acuáticos*

Por razones de claridad, la Comisión aceptó la mayoría de los comentarios recibidos sobre la evaluación de la infección por el virus iridiscente de los decápodos tipo 1 con respecto a los criterios de inclusión de las enfermedades de los animales acuáticos en el Artículo 1.2.2 del Capítulo 1.2, *Criterios para la inclusión de las enfermedades de los animales acuáticos*.

La evaluación revisada de la infección por el DIV1 se presenta en el Anexo 10 para información de los Miembros (véase también ítem 7.1.2).

8. *MANUAL DE LAS PRUEBAS DE DIAGNÓSTICO PARA LOS ANIMALES ACUÁTICOS*

8.1. Situación de las revisiones del *Manual Acuático*

La Comisión para los Animales Acuáticos examinó la situación de los capítulos que se habían identificado previamente para una profunda revisión. Estos capítulos incluyen los del Título 2.3, *Enfermedades de los peces*, y un nuevo capítulo sobre la infección por *Batrachochytrium salamandrivorans*. En la reunión de septiembre de 2020, la Comisión prevé distribuir para examen los primeros borradores de los capítulos restantes sobre enfermedades de los peces, que se han revisado utilizando el nuevo modelo. La Comisión reiteró la decisión de no seguir revisando los capítulos sobre las enfermedades retiradas de la lista y de suprimirlos del *Manual Acuático* después de la Sesión General de mayo de 2021. La Comisión señaló la importancia de concentrar los recursos disponibles en mantener la calidad de los capítulos de las enfermedades de la lista. Los capítulos de enfermedades retiradas de la lista seguirán disponibles en versiones previas del *Manual Acuático*.

8.2. Textos propuestos para adopción en la 88.ª Sesión General en mayo de 2021

Modificaciones horizontales

La Comisión para los Animales Acuáticos aceptó añadir "aparentemente" en el título de la Tabla 4.1, *Métodos de diagnóstico recomendados por la OIE y su nivel de validación para la vigilancia de animales sanos y la investigación de animales clínicamente afectados*, antes de "animales sanos". Esta modificación se aplicará de forma horizontal en todos los capítulos del *Manual Acuático* y en el modelo.

La Comisión rechazó la combinación de los métodos de prueba en las líneas de la Tabla 4.1, por ejemplo, combinar el cultivo celular y un método molecular o inmunológico en la misma línea. La Comisión recordó a los Miembros que los métodos de prueba se enumeran por separado en la Tabla 4.1 y que las combinaciones de métodos de prueba, necesarias para definir un caso sospechoso o confirmado, figuran en la Sección 6, *Criterios de diagnóstico confirmativo*.

En respuesta a una serie de comentarios relativos al uso de abreviaturas, la Comisión reiteró que, en el *Manual Acuático*, "RT-PCR" se reserva para los métodos de reacción en cadena de la polimerasa de transcripción inversa; la "PCR en tiempo real" siempre se indica en su totalidad. El término "PCR convencional" se utiliza en todo el *Manual Acuático*, incluso en la Tabla 4.1 y en el título de la Sección 4.4.2.

La Comisión aceptó la supresión del término "serológicos" del título de la Sección 4.10, *Otros métodos serológicos*, ya que los otros métodos no deben limitarse a los serológicos. Esta modificación se aplicará de forma horizontal en todos los capítulos del *Manual Acuático* y en el modelo.

La Comisión no estuvo de acuerdo con una serie de cambios propuestos al texto de la Sección 6, *Criterios de diagnóstico confirmativo*. El texto de esta sección figura en el modelo de capítulo y se aplica como norma a todos los demás.

La Comisión estuvo de acuerdo con añadir una explicación sobre la finalidad de la Tabla 6.1, Realización de pruebas recomendadas para la vigilancia o el diagnóstico, que figura en la Sección 6.3, *Sensibilidad y especificidad de las pruebas de diagnóstico*. Esta modificación se aplicará de forma horizontal en todos los capítulos del *Manual Acuático* y en el modelo. La Comisión reiteró que la información incluida en el cuadro es necesaria para interpretar los resultados de las pruebas; cuando no se disponga de información para completar la tabla (debido a la falta de estimaciones de la sensibilidad y especificidad del diagnóstico), se indicará en esta sección.

8.2.1. Infección por el virus de la viremia primaveral de la carpa (Capítulo 2.3.9)

Se recibieron comentarios de Australia, Canadá, China (Rep. Pop.), Corea (Rep.), Singapur, Taipéi Chino, Tailandia, la UE y AU-IBAR.

La Comisión para los Animales Acuáticos no estuvo de acuerdo con un comentario en el que se proponía suprimir el tritón vientre de fuego (*Cynops* spp.) y el camarón patiblanco *(Penaeus vannamei)*, de la Sección 2.2.2, *Especies con evidencia incompleta susceptibilidad*, aduciendo que no eran especies de peces de aleta. La Comisión tomó nota de que el Grupo *ad hoc* sobre la susceptibilidad de las especies de peces a la infección por las enfermedades de la lista de la OIE había determinado que esas especies cumplían los criterios de inclusión en esta sección. La Comisión subrayó que la finalidad de esta sección era proporcionar información pertinente para la evaluación del riesgo y destacar las cuestiones que pudieran justificar futuras investigaciones.

La Comisión no estuvo de acuerdo con desplazar la trucha arco iris (*Oncorhynchus mykiss*) de la Sección 2.2.2, Especies con evidencia incompleta susceptibilidad, a la sección 2.2.1, *Especies hospedadoras susceptibles*, ya que el País Miembro no había aportado ninguna prueba nueva que justificara un nuevo examen de las conclusiones del grupo *ad hoc*.

En respuesta al comentario de un País Miembro de añadir información más reciente a la Sección 2.4.6, *Desinfección de huevos y larvas*, la Comisión confirmó que la referencia actual es válida y que no tenía conocimiento de ningún estudio científico más reciente sobre el tema.

En respuesta a una solicitud de ampliar los datos brindados en la Sección 3.5.3, *Muestras fijas para histopatología, inmunohistoquímica o hibridación* in situ, la Comisión indicó que la información genérica sobre las técnicas de fijación figura en el Capítulo 2.3.0, *Información general*. No se aportó ningún fundamento para repetir la información en este capítulo ni brindar orientaciones específicas para el virus de la viremia primaveral de la carpa.

Un País Miembro propuso añadir una frase a la Sección 3.6, *Agrupación de muestras*, refiriéndose a la necesidad de considerar el impacto de la agrupación en las decisiones relacionadas con el comercio. La Comisión observó que, dado que el *Manual Acuático* ofrece sobre todo métodos de pruebas de diagnóstico, no se trata del texto apropiado para disposiciones referidas al comercio.

En cuanto a la Tabla 4.1, en el que la OIE recomienda los métodos de diagnóstico y su nivel de validación para la vigilancia de animales sanos y la investigación de animales clínicamente afectados, un Miembro propuso que se aumentara de 1 a 3 el nivel de validación para el cultivo de células dado que se utilizaba desde hace muchos años. La Comisión tomó nota de que los niveles de validación se basan en estudios publicados en revistas revisadas por pares y están en conformidad con el proceso de validación de la OIE que figura en el Capítulo 1.1.2, *Principios y métodos de validación de las pruebas de diagnóstico de las enfermedades infecciosas*.

La Comisión no estuvo de acuerdo con añadir una frase a la Sección 4.3, *Cultivo de células o medios artificiales para el aislamiento*, que explique que una prueba puede ser declarada negativa si no se observa un efecto citopático (CPE), debido al riesgo de obtener resultados falsos positivos por CPE poco claros o atípicos.

La Comisión no aceptó incluir un párrafo en la Sección 5, *Prueba(s) recomendada(s) para la vigilancia para demostrar la ausencia de enfermedad en poblaciones aparentemente sanas*, indicando que las pruebas descritas en el capítulo no bastan para lograr la ausencia de enfermedad. Una vez más, el *Manual Acuático* no es el texto apropiado para tales declaraciones.

El Capítulo 2.3.9 revisado, *Infección por el virus de la viremia primaveral de la carpa*, figura en el Anexo 11 para comentario de los Miembros. La aprobación del capítulo revisado se ha pospuesto hasta la 89.ª Sesión General de mayo de 2021. Sabiendo que el capítulo ya ha sido objeto de amplias consultas, se solicita a los Miembros que sólo presenten observaciones para abordar cuestiones de fondo que no hayan sido examinadas anteriormente.

8.2.2. Infección por *Batrachochytrium salamandrivorans* (Capítulo 2.1.3)

Se recibieron comentarios de Australia, Canadá, China (Rep. Pop.), Tailandia, la UE y AU-IBAR.

Se recibieron varios comentarios editoriales menores y el capítulo se modificó en consecuencia.

En la Tabla 4.1, métodos de diagnóstico recomendados por la OIE y su nivel de validación para la vigilancia de animales sanos y la investigación de animales clínicamente afectados, la Comisión para los Animales Acuáticos aceptó eliminar la prueba del flujo lateral de la columna A, Vigilancia de animales aparentemente sanos, y bajar su calificación en la columna B, Diagnóstico presunto de animales clínicamente afectados, debido a la baja especificidad del método. En consecuencia, se suprimió el método de la Sección 6.1.1, *Definición de caso sospechoso en animales aparentemente sanos.*

El Capítulo 2.1.3 revisado, Infección por *Batrachochytrium salamandrivorans*, figura en el Anexo 12 para comentario de los Miembros. La aprobación del capítulo revisado se ha pospuesto hasta la 89.ª Sesión General de mayo de 2021. Sabiendo que el capítulo ya ha sido objeto de amplias consultas, se pide a los Miembros que sólo presenten observaciones para abordar cuestiones de fondo que no hayan sido examinadas anteriormente.

8.2.3. Infección por el virus de la necrosis hematopoyética infecciosa (Capítulo 2.3.4)

Se recibieron comentarios de Canadá, China (Rep. Pop.), Corea (Rep.), Tailandia, la UE y AU-IBAR.

Con fines de coherencia, la Comisión para los Animales Acuáticos revisó este capítulo junto con el Capítulo 2.3.10, *Infección por el virus de la septicemia hemorrágica viral*, debido a las similitudes entre ambos capítulos.

La Comisión convino en ampliar el alcance geográfico de la infección por el virus de la necrosis hematopoyética infecciosa para incluir a África y añadir la referencia publicada al capítulo.

La Comisión modificó una propuesta de enmienda en la Sección 2.3.4, *Modos de transmisión y ciclo de vida*, y así establecer claramente que no existen pruebas suficientes para demostrar la verdadera transmisión vertical de la enfermedad y explicitar el papel de la desinfección en la prevención de la transmisión.

Se rechazó la propuesta de añadir "del *Manual Acuático*" después de "2.4" en la frase "Esta sección se basa en la información de las secciones 2.2, 2.3 y 2.4 para identificar las poblaciones, los individuos y las muestras que tienen más probabilidades de estar infectados", ya que las secciones mencionadas se encuentran en del mismo capítulo.

En la Sección 3.5, *Conservación de las muestras para su envío*, un País Miembro solicitó se borrara una referencia al Capítulo 2.3.0, *Información general*, indicando que el capítulo no proporciona detalles sobre la preservación de las muestras virales. La Comisión señaló que en la Sección 2.2, *Examen virológico*, del Capítulo 2.3.0 se describe el transporte y el tratamiento de las muestras con antibiótico para el examen virológico y, por consiguiente, llegó a la conclusión de que era apropiado mantener la referencia al Capítulo 2.3.0.

La Comisión aceptó la propuesta de añadir el texto estándar a la Sección 3.6, *Combinación de varias muestras*. Sin embargo, no estuvo de acuerdo en que se incluyera una frase sobre la necesidad de examinar el impacto del intercambio en decisiones relacionadas con el comercio. La Comisión reafirmó que, dado que el *Manual Acuático* proporciona principalmente métodos de pruebas de diagnóstico, no es el adecuado para tratar disposiciones sobre el comercio.

La Comisión examinó y enmendó o añadió algunos de los niveles de validación que figuran en la Tabla 4.1, Métodos de diagnóstico recomendados por la OIE y su nivel de validación para la vigilancia de los animales sanos e investigación de los animales infectados clínicamente.

El Capítulo 2.3.4 revisado, *Infección por el virus de la necrosis hematopoyética infecciosa*, figura en el Anexo 13 para comentario de los Miembros. La aprobación del capítulo revisado se ha pospuesto hasta la 89.ª Sesión General de mayo de 2021. Sabiendo que el capítulo ya ha sido objeto de amplias consultas, se pide a los Miembros que sólo presenten observaciones para abordar cuestiones de fondo que no hayan sido examinadas anteriormente.

8.2.4. Infección por el virus de la septicemia hemorrágica viral (Capítulo 2.3.10)

El capítulo se actualizó con la ayuda de los tres laboratorios de referencia de la OIE para el virus de la septicemia hemorrágica viral. Se recibieron comentarios de Canadá, China (Rep. Pop.), Corea (Rep. de), Tailandia, la UE y UA-IBAR.

Con fines de coherencia, la Comisión para los Animales Acuáticos revisó este capítulo junto con el Capítulo 2.3.4, *Infección por el virus de la necrosis hematopoyética infecciosa*, debido a las similitudes entre ambos capítulos.

Un País Miembro propuso que se modificara la Sección 2.2.1, *Especies hospedadoras susceptibles*, para señalar la susceptibilidad de *Clupea harengus* (arenque del Atlántico) al genotipo IVa (además del genotipo Ib y III). Tras examinar la información científica, la Comisión concluyó que no estaba de acuerdo con la propuesta, ya que existían pruebas incompletas de la susceptibilidad de *C. harengus* al genotipo IVa de la infección por el virus de la septicemia hemorrágica viral. La Comisión también comunicó a los Miembros que no hay diferenciación de genotipos para la infección por el virus de la septicemia hemorrágica viral y que, por consiguiente, *C. harengus* debía suprimirse del cuadro de la Sección 2.2.2.

La Comisión no aceptó añadir *Salvelinus fontinalis* y *Lota lota* a la Sección 2.2.1, dado que la solicitud no estaba fundamentada por información científica publicada.

La Comisión no estuvo de acuerdo con un comentario que sugería en inglés el cambio del nombre común de *Paralichthys olivaceus* por el de "*olive flounder*", Si bien reconoció que se trataba de un nombre más aceptable, recuerda a los Miembros que utiliza los nombres comunes indicados para las especies en la base de datos terminológica de la FAO.

La Comisión denegó suprimir el texto de la Sección 2.4.4, ya que contiene información pertinente y útil.

La Comisión no estuvo de acuerdo con las enmiendas propuestas a la Sección 3.1, *Selección de poblaciones y ejemplares individuales*, ya que el texto proporciona una orientación útil. La sección se armonizó con la sección correspondiente del Capítulo 2.3.4.

No se aceptó borrar texto de los párrafos de la Sección 3.5.1, *Muestras para el aislamiento de patógenos*, ya que no se proporcionó ninguna justificación y el texto se consideró de interés.

Los comentarios técnicos relativos a la Sección 4.4, *Amplificación de ácido nucleico*, y la Sección 4.9.2, *Prueba de anticuerpos fluorescentes indirectos*, se remitieron a los expertos del laboratorio de referencia de la OIE.

En la Sección 4.4.1, *RT-PCR en tiempo real*, la Comisión no estuvo de acuerdo con eliminar la mención a un kit comercial, ya que la prueba se había validado utilizando el kit. Sin embargo, la Comisión señaló que prefería no hacer referencia a los productos comerciales a menos que se justifique una mención específica.

La Comisión no estuvo de acuerdo en añadir en que la prueba no se había validado en la Sección 4.7, *RT-PCR en tiempo real*, dado que los resultados del diagnóstico se habían evaluado parcialmente y que el rendimiento de la prueba se había comparado con el del cultivo celular.

El Capítulo 2.3.10 revisado, *Infección por el virus de la septicemia hemorrágica viral*, figura en el Anexo 14 para comentario de los Miembros. La aprobación del capítulo revisado se ha pospuesto hasta la 89.ª Sesión General de mayo de 2021. Sabiendo que el capítulo ya ha sido objeto de amplias consultas, se pide a los Miembros que sólo presenten observaciones para abordar cuestiones de fondo que no hayan sido examinadas anteriormente.

8.3. Textos para comentario de los Miembros

Se recordó a los Miembros que la Comisión para los Animales Acuáticos había comenzado a formatear progresivamente los capítulos específicos de enfermedad del *Manual Acuático* utilizando el nuevo modelo. Sabiendo que los capítulos reformateados y actualizados tienen cambios sustanciales, en la última reunión de septiembre de 2019, la Comisión explicó que en el informe sólo se proporcionarían versiones limpias de los capítulos. Los cambios posteriores introducidos en estas revisiones iniciales tras los comentarios de los Miembros se indicarán de la forma habitual (es decir, ~~tachado~~ para el texto borrado y doble subrayado para el texto añadido).

Se creará un documento con la comparación entre la versión adoptada de un capítulo y el nuevo texto propuesto. Este documento de comparación no se incluirá en el informe de la Comisión, pero se podrá solicitar al Departamento de Normas de la OIE (standards.dept@oie.int).

8.3.1. Infección por *Gyrodactylus salaris* (Capítulo 2.3.3)

La Comisión examinó el Capítulo 2.3.3, Infección por *Gyrodactylus salaris*, que se había actualizado con la ayuda del experto del laboratorio de referencia de la OIE y modificado a partir del nuevo modelo del capítulo específico de enfermedad. Entre las principales modificaciones, figuran un texto explicativo sobre la taxonomía de *G. thymalli* y *G. salaris* y el enfoque adoptado en el *Manual Acuático* para distinguir estas especies; una sección actualizada sobre la selección de especímenes y la toma de muestras, el transporte y la manipulación; la inclusión de un método de DNA ambiental para la detección de *G. salaris*; y definiciones revisadas de los casos sospechosos y confirmados en animales aparentemente sanos y clínicamente afectados.

El Capítulo 2.3.3 revisado, Infección por *Gyrodactylus salaris*, figura en el Anexo 15 para comentario de los Miembros.

8.3.2. Infección por el alfavirus de los salmónidos (Capítulo 2.3.6)

La Comisión examinó el Capítulo 2.3.6, *Infección por el alfavirus de los salmónidos*, que había sido actualizado por el experto del laboratorio de referencia de la OIE y modificado a partir del nuevo modelo del capítulo específico de enfermedad. Entre las principales enmiendas figuran las secciones actualizadas sobre factores del hospedador; patrón de la enfermedad; selección de especímenes, toma de muestras, transporte y manipulación; texto actualizado sobre histopatología y citopatología, y definiciones revisadas de casos sospechosos y confirmados en animales aparentemente sanos y clínicamente afectados.

El Capítulo 2.3.6 revisado, *Infección por el alfavirus de los salmónidos*, figura en el Anexo 16 para comentario de los Miembros.

8.4. Textos para información de los Países Miembros

8.4.1. Actualización de los capítulos de introducción para cada grupo de hospedadores: anfibios, crustáceos, peces y moluscos (Capítulos 2.1.0, 2.2.0, 2.3.0 y 2.4.0 respectivamente)

La Comisión para los Animales Acuáticos identificó la necesidad de actualizar los capítulos introductorios de información general para cada especie hospedadora. El Capítulo 2.3.0, *Información general*, destinado a las enfermedades de los peces, será el primer capítulo que se actualice. Para esta revisión, la Comisión solicitará la ayuda de todos los expertos de los laboratorios de referencia de la OIE para las enfermedades de los peces. Se solicitará a los expertos que designen un encargado, que recabará las conclusiones del grupo para que el texto sea lo más completo e informativo posible. La Comisión prevé la difusión de un Capítulo 2.3.0 revisado en su informe de septiembre de 2020.

8.4.2. Utilización de los métodos de ADN ambiental para la vigilancia de las enfermedades de los animales acuáticos

La vigilancia de los sistemas acuáticos mediante el uso de ADN ambiental es un campo de investigación que avanza rápidamente y que brindará oportunidades a la hora de aplicar métodos rápidos, rentables y no invasivos para la detección de patógenos, especialmente en las poblaciones acuáticas silvestres, donde puede resultar difícil o inapropiado realizar una toma de muestras. La Comisión para los Animales Acuáticos es consciente de que existen métodos de ADN ambiental destinados a detectar agentes patógenos de varias de las enfermedades de la lista, entre ellos, *Xenohaliotis californiensis, Batrachochytrium dendrobatidis, Aphanomyces astaci y Gyrodactylus salaris.*

La Comisión acordó que, dado que estos métodos están disponibles y se utilizan en la actualidad, se recomienda brindar información sobre su correcta aplicación, así como las posibles limitaciones. La Comisión observó que, ante la falta de estimaciones precisas de los resultados del diagnóstico para diseñar programas de vigilancia que utilicen eDNA (ADN ambiental), los datos obtenidos con este método tal vez no sean adecuados para justificar la declaración de ausencia de enfermedades de la lista. Si bien la Comisión observó que la confirmación de la infección para las enfermedades de la lista no podía hacerse utilizando los métodos de ADN ambiental; los resultados positivos podían considerarse criterios apropiados para un caso sospechoso.

La Comisión acordó elaborar un documento de orientación destacando las consideraciones para un uso apropiado, los beneficios y las restricciones de los métodos de ADN ambiental. El documento busca orientar el uso apropiado de estos métodos y será examinado por la Comisión en su próxima reunión de septiembre de 2020. Se propone el uso de un método de ADN ambiental para la detección de *Gyrodactylus salaris* para su inclusión en el capítulo del *Manual Acuático* relativo a la infección por *Gyrodactylus salaris* (véase el Anexo 15 para comentario de los Miembros).

9. GRUPOS *AD HOC* DE LA OIE

9.1. Grupo *ad hoc* sobre el virus de la tilapia del lago

Se informó a la Comisión de Animales Acuáticos de que el Grupo *ad hoc* sobre el virus de la tilapia del lago (TiLV) seguía trabajando en la evaluación de las pruebas de diagnóstico disponibles para el TiLV y presentará un informe final en la reunión de la Comisión de septiembre de 2020.

9.2. Grupo *ad hoc* sobre la susceptibilidad de las especies de moluscos a la infección por enfermedades de la lista de la OIE

Se informó a la Comisión de que el Grupo *ad hoc* sobre la susceptibilidad de las especies de moluscos a la infección por las enfermedades de la lista de la OIE se había reunido por primera vez en enero de 2020 con el fin de iniciar los trabajos sobre la aplicación del Capítulo 1.5, *Criterios para la inclusión de especies susceptibles de infección por un agente patógeno específico*, a las enfermedades de los moluscos de la lista de la OIE. El grupo *ad hoc* se reunirá nuevamente en junio de 2020 para continuar su labor. La Comisión agradeció al grupo *ad hoc* el trabajo realizado hasta la fecha.

10. CENTROS DE REFERENCIA DE LA OIE O CAMBIO DE EXPERTOS

10.1. Evaluación de las solicitudes para la designación de centros de referencia de la OIE en temas relativos a la sanidad de los animales acuáticos o el cambio de expertos.

La Comisión recomendó se acepten las siguientes solicitudes para la designación como laboratorio de referencia de la OIE:

Laboratorio de referencia de la OIE para la enfermedad de la necrosis hepatopancreática aguda
Aquaculture Pathology Laboratory, School of Animal and Comparative Biomedical Sciences, University of Arizona, 1117 E Lowell St., Tucson, Arizona 85721, ESTADOS UNIDOS DE AMÉRICA
Tel: +1-520 621 4438
Email: aquapath@cals.arizona.edu; adhar@email.arizona.edu

Sitio web: www.aquapath.lab.arizona.edu

Experto de referencia designado: Dr. Arun Dhar

Laboratorio de referencia de la OIE para el virus del síndrome de las manchas blancas
Aquaculture Pathology Laboratory, School of Animal and Comparative Biomedical Sciences, University of Arizona, 1117 E Lowell St., Tucson, Arizona 85721, ESTADOS UNIDOS DE AMÉRICA
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Experto de referencia designado: Dr. Arun Dhar

Laboratorio de referencia de la OIE para la necrosis hipodérmica y hematopoyética infecciosa
Aquaculture Pathology Laboratory, School of Animal and Comparative Biomedical Sciences, University of Arizona, 1117 E Lowell St., Tucson, Arizona 85721, ESTADOS UNIDOS DE AMÉRICA
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Sitio web: www.aquapath.lab.arizona.edu

Experto de referencia designado: Dr. Arun Dhar

Laboratorio de referencia de la OIE para Hepatobacter penaei (*hepatopancreatitis necrotizante*)
Aquaculture Pathology Laboratory, School of Animal and Comparative Biomedical Sciences, University of Arizona, 1117 E Lowell St., Tucson, Arizona 85721, ESTADOS UNIDOS DE AMÉRICA
Tel: +1-520 621 4438
Email: lfarangu@email.arizona.edu

Sitio web: www.aquapath.lab.arizona.edu

Experto de referencia designado: Dr. Luis Fernando Aranguren

10.2. Evaluación de los informes anuales de los laboratorios de referencia de la OIE

Se recibieron los informes anuales de todos los laboratorios de referencia de la OIE para las enfermedades de los animales acuáticos, excepto uno, y de todos los centros colaboradores para las cuestiones relativas a los animales acuáticos. Se enviará una carta al laboratorio que no presentó el informe recordándole al experto sus obligaciones y solicitándole que presente un informe para su examen en la reunión de septiembre de 2020 de la Comisión para los Animales Acuáticos.

De acuerdo con el texto aprobado de los *Procedimientos para la designación de los laboratorios de referencia de la OIE* (https://www.oie.int/es/nuestra-experiencia-cientifica/laboratorios-de-referencia/sops/) y los *Procedimientos para la designación de los centros colaboradores de la OIE (*https://www.oie.int/es/nuestra-experiencia-cientifica/centros-colaboradores/sops/), la Comisión revisó todos los informes recibidos, en particular el desempeño de cada centro de referencia con respecto al cumplimiento del mandato para beneficio de los Miembros de la OIE.

La Comisión destaca la importante contribución de los laboratorios de referencia en 2019 y agradece a los expertos designados por dirigir estas valiosas contribuciones para la misión de la OIE. No obstante, la Comisión identificó un laboratorio de referencia que no cumplía con el mandato principal y se solicitará al laboratorio en cuestión que explique su situación y las posibles razones de la ausencia de actividad; el Delegado recibirá una copia de toda la correspondencia.

La Comisión expresó su agradecimiento por el apoyo entusiasta y el asesoramiento prestado a la OIE por los centros de referencia.

10.3. Evaluación de los planes de trabajo quinquenales de los centros colaboradores de la OIE para las enfermedades de los animales acuáticos

La Comisión para los Animales Acuáticos examinó los planes de trabajo quinquenales enviados por dos de los tres centros colaboradores. La Comisión quedó impresionada por la gama de actividades y su pertinencia y coherencia con la estrategia de la OIE en materia de sanidad de los animales acuáticos. La Comisión proporcionó información sobre la utilización del modelo de plan de trabajo a uno de estos centros colaboradores. Se solicitará al centro restante que presente un plan de trabajo para su revisión en la próxima reunión de la Comisión en septiembre de 2020.

11. OTROS TEMAS

11.1 Mejora de los procedimientos operativos estándar para el registro de los kits de diagnóstico certificados por la OIE

La secretaría de la OIE para el registro de kits de diagnóstico presentó una breve reseña de su procedimiento de registro y describió algunos cambios introducidos recientemente en aras de claridad y transparencia al procedimiento propiamente dicho y con vistas a normalizar la preparación de informes.

La secretaría también preparó un proyecto de revisión de los POE para el registro de los kits de diagnóstico de la OIE, en el que se describen dichos cambios y se brinda más información de contexto sobre los requisitos de registro correspondientes. Los POE se presentaron a la aprobación de la Comisión para validación.

12. FECHA DE LA PRÓXIMA REUNIÓN

La próxima reunión de la Comisión para los Animales Acuáticos se llevará a cabo del 26 agosto al 2 de septiembre de 2020.

Anexo 1

INFORME DE LA REUNIÓN DE LA COMISIÓN DE NORMAS SANITARIAS PARA LOS ANIMALES ACUÁTICOS DE LA OIE

París, 19-26 de febrero de 2020

Lista de participantes

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Anexo 2

CAPÍTULO 4.X.

BIOSEGURIDAD PARA LOS ESTABLECIMIENTOS DE ACUICULTURA

Artículo 4.X.1.

Finalidad

Brindar recomendaciones sobre el desarrollo y la implementación de medidas de *bioseguridad* destinadas principalmente a mitigar el *riesgo* de introducción de *agentes patógenos* específicos en los *establecimientos de acuicultura*, y si se introducen *agentes patógenos*, mitigar el *riesgo* de una mayor propagación dentro, o de liberación desde los *establecimientos de acuicultura*.

Artículo 4.X.2.

Ámbito de aplicación

Los principios de *bioseguridad* son pertinentes para la aplicación de las normas del *Código Acuático* a nivel de un país, *zona*, *compartimento* o *establecimiento de acuicultura*, ~~según corresponda~~. Este capítulo describe las recomendaciones sobre *bioseguridad* que se deberán aplicar en los *establecimientos de acuicultura,* incluidos los sistemas semi-abiertos semi-cerrados y cerrados. El capítulo describe los principios generales para planificar la *bioseguridad,* las categorías de los sistemas de producción en *acuicultura*, ~~las principales rutas de transmisión,~~ las medidas de mitigación para las rutas de transmisión, ~~el uso~~ la aplicación del *análisis del riesgo* y los enfoques para desarrollar un *plan de bioseguridad* ~~y los componentes clave de un plan~~.

Para más información sobre la prevención y el control de las *enfermedades*, consulte el Título 4 del *Código Acuático*.

Artículo 4.X.3.

Introducción

~~La aplicación de las medidas de *bioseguridad* constituye el principio fundamental que sustenta la prevención de las *enfermedades* de los *animales acuáticos* a nivel del país, la *zona* o el *compartimento*.~~ La *bioseguridad* a nivel de un *establecimiento de acuicultura* es parte integrante de una *bioseguridad* efectiva a escala de un país, una *zona* o un *compartimento* y, por ende, para un estatus sanitario y un bienestar óptimos de las poblaciones de *animales acuáticos*. Este capítulo describe los principios de *bioseguridad* diseñados para mitigar los *riesgos* asociados con la introducción, la propagación dentro, o la liberación de *agentes patógenos* desde los *establecimientos de acuicultura* ~~y para mantener la sanidad óptima de sus poblaciones de *animales acuáticos*~~.

Dados los desafíos únicos que plantea la gran diversidad de sistemas de producción acuícola y la amplia diversidad de especies de *animales acuáticos* de cría, el desarrollo de *planes de bioseguridad* para los *establecimientos de acuicultura* requiere la evaluación de los *riesgos* de *enfermedad* planteados por *agentes patógenos* específicos y sus posibles rutas de transmisión. Un *plan de bioseguridad* describe las medidas físicas y de gestión destinadas a mitigar los *riesgos* identificados de acuerdo con las circunstancias particulares del *establecimiento de acuicultura*. El personal de los *establecimientos de acuicultura*, ~~y~~ los proveedores de servicios ~~y los veterinarios~~ y los *profesionales de sanidad de los animales acuáticos* o los *veterinarios* deberán comprometerse con el desarrollo y la implementación del *plan de bioseguridad* para garantizar que sea práctico y eficaz.

El resultado alcanzado a través de la implementación de la *bioseguridad* en los *establecimientos de acuicultura* es una mejor sanidad y bienestar de los *animales acuáticos* durante todo el ciclo de producción. Entre los beneficios pueden figura~~n~~r una mejora del acceso a los mercados y una mayor productividad, de forma directa por medio de unas mayores tasas de supervivencia, índices de crecimiento y conversión alimentaria y, de forma indirecta, a través de ~~la~~ una reducción en el uso ~~de los tratamientos~~ de productos médicos veterinarios (incluyendo *agentes antimicrobianos)* y de los costos de producción asociados.

Artículo 4.X.4.

Principios generales

La *bioseguridad* es una serie de medidas físicas y de gestión que, empleadas en su conjunto, reducen de forma acumulativa el *riesgo* de *infección* en las poblaciones de *animales acuáticos* ~~en~~ dentro de un *establecimiento de acuicultura*. La planificación e implementación de la *bioseguridad* en un *establecimiento de acuicultura* requieren ~~planificación para~~ identificar los *riesgos* y ~~considerar~~ medidas costo-efectivas, con el fin de alcanzar los objetivos identificados en el plan. Las medidas requeridas variarán ~~según~~ entre los *establecimientos de acuicultura* dependiendo de factores como ~~el *riesgo*~~ la probabilidad de exposición a los *agentes patógenos*, ~~las especies de~~ los *animales acuáticos* de cría, la categoría del sistema de producción acuícola, las prácticas de cultivo y la situación geográfica. Si bien se pueden utilizar distintos enfoques con miras a alcanzar un objetivo identificado de bioseguridad, ~~a continuación,~~ ~~se describen~~ los principios generales para desarrollar e implementar un *plan de bioseguridad* son coherentes y se describen a continuación:

~~1) Planificación para documentar el objetivo del *plan de seguridad*, los *riesgos* identificados que se gestionarán, las medidas que se implementarán para afrontar los *riesgos* de *enfermedad*, los procedimientos operativos y de seguimiento requeridos, que se describen en los Artículos 4.X.6. y 4.X.7.;~~

1~~2~~) Identificación de las posibles rutas de transmisión de los *agentes patógenos* en, propagación dentro y liberación desde el *establecimiento de acuicultura*, que se describen en el ~~los~~ Artículo~~s~~ ~~4.X.5. y~~ 4.X.6., teniendo en cuenta el tipo de sistema de producción y el diseño del *establecimiento de acuicultura*.

2~~3~~) Realización de un *análisis del riesgo* para identificar y evaluar las amenazas de ~~*bioseguridad*~~ *enfermedad* y garantizar que el plan cubra los *riesgos* de forma apropiada y eficaz. El *análisis de riesgo* puede variar de un análisis simple a uno complejo, en función de los objetivos del *plan de bioseguridad*, ~~y~~ de las circunstancias del *establecimiento de acuicultura* y de los *riesgos* de *enfermedad*, como se describen en el Artículo 4.X.7.

3~~4~~) Evaluación de las medidas de *bioseguridad* para tratar los *riesgos* de *enfermedad* identificados a partir de su eficacia potencial, costos iniciales y en curso (por ejemplo, remodelación de edificios, mantenimiento) y de los requisitos de gestión, que se describen en el Artículo 4.X.7.

4~~5~~) Integración de las prácticas de gestión en los procedimientos operativos de los *establecimientos de acuicultura* y formación del personal relevante sobre ~~asociada con~~ dichos procedimientos, como se describe en los Artículos 4.X.7. y 4.X.8.

5) Señalización clara que debe exponerse para promover la sensibilización y el cumplimiento con las medidas del *plan de bioseguridad* por parte del personal, los visitantes y el público.

6~~5~~) Registros y documentación apropiados que resultan esenciales para demostrar la implementación efectiva del *plan de bioseguridad*. En el Artículo 4.X.8. se ofrecen algunos ejemplos.

7~~6~~) ~~Revisión de rutina del~~ Descripción del calendario para las revisiones de rutina y las auditorías del *plan de bioseguridad*. ~~e identificación de~~ Se han de determinar los factores desencadenantes de una revisión (por ejemplo, *brotes* de *enfermedad*, cambios en la infraestructura, técnicas de producción, ~~*brotes de enfermedad*,~~ o perfiles de *riesgo*). Se pueden requerir ~~una~~ auditorías externas cuando clientes, o entidades reguladores o razones de acceso al mercado exigen el reconocimiento de las medidas de *bioseguridad*, ~~como se describe en el~~ de acuerdo con las disposiciones del Artículo 4.X.8.

Artículo 4.X.5.

Categorías de los sistemas de producción acuícola

~~Los *animales acuáticos* se pueden producir en~~ Se definen c~~C~~uatro categorías diferentes de sistemas de producción~~, definidos~~ en función de la capacidad de tratar el agua que entra y sale del sistema, y del nivel de control ~~de~~ sobre los *animales acuáticos* y ~~de~~ los *vectores*. ~~Estas medidas~~ Estos factores necesitan considerarse en el marco de la elaboración de un *plan de bioseguridad*.

Sistemas abiertos

~~Los~~ En un sistema~~s~~ de producción de *acuicultura* abierto~~s~~ no es posible controlar el ~~tienen ningún control del~~ agua, ni ~~de~~ las condiciones medioambientales~~ o~~, los animales ~~ni~~ o los *vectores*. Estos sistemas de producción pueden incluir el aumento de las existencias de las poblaciones silvestres con *animales acuáticos* provenientes de *establecimientos de acuicultura* o del entorno silvestre. Dado que no pueden ser considerados "*establecimientos de acuicultura*", no se toman en cuenta en este capítulo. Sin embargo, el transporte de los *animales acuáticos* de *establecimientos de acuicultura* a ~~los~~ sistemas abiertos deberá evaluarse para determinar la necesidad de ~~estar sujeto a las~~ medidas de mitigación de *enfermedad*.

Sistemas semi-abiertos

En un sistema de producción de *acuicultura* semi-abierto, no es posible controlar el agua que entra o sale del sistema o las condiciones medioambientales. Algunos *animales acuáticos* y *vectores* también pueden entrar y salir del sistema. Los cercados de malla para los peces de aleta y las jaulas suspendidas para ~~de *acuicultura* de~~ moluscos en cuerpos de agua naturales ~~y la *acuicultura* de moluscos suspendidos en columnas de agua o en el piso del océano~~ son ejemplos de sistemas de producción de *acuicultura* semi-abiertos.

Sistemas semi-cerrados

En un sistema de producción de *acuicultura* semi-cerrado, existe cierto control sobre ~~d~~el agua que entra y sale del sistema y ~~de~~ sobre las condiciones medioambientales. Si bien puede prevenirse la entrada y salida de los *animales acuáticos* y de los *vectores* en el sistema, existe un control limitado para prevenir la entrada o la salida de los *agentes patógenos*. Los estanques, canales de flujo continuo, jaulas flotantes cerradas y tanques con ~~re~~circulación de agua son ejemplos de sistemas de producción de *acuicultura* semi-cerrados.

Sistemas cerrados

En un sistema de producción de *acuicultura* cerrado, hay suficiente ~~el~~ control del agua que entra y sale del sistema ~~permite~~ para excluir *animales acuáticos*, *vectores* y *agentes patógenos*. Las condiciones ambientales también se pueden controlar. Los sistemas de recirculación en la producción de *acuicultura*, los sistemas de producción con un suministro seguro de agua libre de *agentes patógenos* o de *animales acuáticos* (por ejemplo, agua subterránea) o aquellos con altos niveles de tratamiento (y redundancia) del agua que entra o sale en el sistema son ejemplos de sistemas de *acuicultura* cerrados. Igualmente, se pueden controlar las condiciones ambientales.

Artículo 4.X.5.bis

Manejo por áreas

Quizá no sea posible controlar la transmisión de *agentes patógenos* entre *establecimientos de acuicultura* semi-abiertos o semi-cerrados situados a proximidad dentro de cuerpos de aguas compartidas. En estas circunstancias, todos los *establecimientos de acuicultura* que se consideran epidemiológicamente vinculados deberán aplicar un conjunto coherente de medidas de *bioseguridad*. Los acuerdos de gestión por áreas pueden formalizar la coordinación de las medidas de *bioseguridad* comunes entre todos los *establecimientos de acuicultura* que se consideran epidemiológicamente vinculados.

Artículo 4.X.6.

Rutas de transmisión~~, y riesgos asociados~~ y medidas de mitigación

Los *agentes patógenos* se pueden desplazar en, propagarse dentro y liberarse desde los *establecimientos de acuicultura* a través de diversas rutas de transmisión. La identificación de todas las rutas de transmisión potenciales es esencial para el desarrollo de un *plan de bioseguridad* eficaz. Se dará prioridad a la mitigación de las rutas que probablemente den lugar a la transmisión de *agentes patógenos* específicos ~~exponen a los *animales acuáticos* susceptibles a altas cargas de *agentes patógenos*~~.

Los *riesgos* asociados con la introducción en, propagación dentro y liberación de los *agentes patógenos* desde un *establecimiento de acuicultura* necesitan considerarse para cada una de las siguientes rutas de transmisión.

1. Animales acuáticos

El desplazamiento de *animales acuáticos* en, dentro y desde los *establecimientos de acuicultura*, ya sea de forma intencional o no, ~~puede plantear~~ ~~suele~~ puede plantear la posibilidad de un alto *riesgo* de transmisión del *agente patógeno*. Este es particularmente el caso cuando se trasladan *animales acuáticos* con infecciones clínicas o subclínicas, o *animales acuáticos* con un estatus sanitario desconocido dentro de una población susceptible.

Los *animales acuáticos* introducidos intencionalmente o desplazados dentro de un *establecimiento de acuicultura*, ~~o que se mueven dentro de él,~~ pueden incluir stock de reproducción, alevines para crecimiento y material genético, como huevos. Para los animales acuáticos, se deberán considerar los mecanismos de transmisión de los *agentes patógenos* tanto horizontales como verticales. El *riesgo* de transmisión de *agentes patógenos* a través de los *animales acuáticos* se ~~puede~~ deberá gestionar~~;~~ tomando en cuenta las siguientes medidas ~~posibles~~ de mitigación ~~incluyen de la siguiente manera~~:

a) Introducir exclusivamente *animales acuáticos* en el *establecimiento de acuicultura* con un estatus sanitario conocido, que sea igual o superior al de los animales del establecimiento.

b) ~~Poner en *cuarentena* a los~~ Si se introducen *animales acuáticos* ~~introducidos~~ con un estatus sanitario desconocido, éstos deberán ponerse en *cuarentena* ~~provenientes de otras poblaciones de cría en unidades de producción separadas o instalaciones especialmente dedicadas a la *cuarentena*~~;

c) Cuando sea apropiado, ~~tratamiento de~~ tratar a los *animales acuáticos* en *cuarentena* para mitigar los *riesgos* de *enfermedad* (por ejemplo, tratamiento de parásitos externos).

d) Garantizar un transporte bioseguro de los *animales acuáticos* que evite la exposición a los *agentes patógenos*.

e) Dentro del establecimiento, sólo desplazar *animales acuáticos* entre las distintas poblaciones tras haber considerado los *riesgos* de *enfermedad* y con vistas a mantener el ~~un alto~~ estatus sanitario más alto posible de la población de *animales acuáticos*.

f) Aislar las poblaciones de *animales acuáticos* que muestran signos clínicos de *enfermedad* de otras poblaciones hasta que la causa se conozca y la situación se resuelva.

g) Retirar los *animales acuáticos* muertos o enfermos de las unidades de producción lo más pronto posible y eliminarlos de forma biosegura de conformidad con el Capítulo 4.7.

h) Notificar mortalidades inexplicables o inusuales, o sospecha de una *enfermedad* de declaración obligatoria de los *animales acuáticos* a la *autoridad competente* de conformidad con los requisitos locales. Se deberá llevar a cabo la investigación y el *diagnóstico* de las causas de la mortalidad.

i) De ser posible, despoblación total de los *establecimientos de acuicultura* por intervalos, por ejemplo, entre generaciones de *animales acuáticos* o ciclos de producción, seguida de limpieza y *desinfección* de las instalaciones de producción. Se deberá proceder al *vacío sanitario* por un periodo suficiente para interrumpir los ciclos de infección y reducir o eliminar el desafío que representa el patógeno para la reintroducción de *animales acuáticos*. El *vacío sanitario* deberá ser coordinado entre *establecimientos de acuicultura* que están epidemiológicamente vinculados a través de cuerpos de aguas compartidas.

j) ~~Cuando sea posible, prevención de los desplazamientos no intencionales de los *animales acuáticos* en, dentro y desde el establecimiento.~~ Considerar medidas físicas para minimizar la probabilidad de que los *animales acuáticos* de cultivo se escapen o la entrada de *animales acuáticos* silvestres en los *establecimientos de acuicultura*. La probabilidad de entrada o escape de *animales acuáticos* será más alta en los sistemas semi-abiertos que en los semi-cerrados.

~~El *riesgo* de desplazamientos no intencionados de *animales acuáticos* estará influenciado por la categoría de sistema de producción acuícola, con una probabilidad más alta en los sistemas semi-cerrados que en los cerrados. Si se encuentra que los *riesgos* son altos, pueden ser necesarias medidas de mitigación físicas.~~

2. Productos y residuos de animales acuáticos

Los *productos de animales acuáticos* también pueden introducirse, desplazarse o retirarse de ~~a~~ un *establecimiento de acuicultura* ~~o circular en él~~; por ejemplo, *productos de animales acuáticos* derivados de *animales acuáticos* criados en otros lugares. Los *residuos de animales acuáticos* pueden generarse cuando ~~incluir el cuerpo entero o partes de~~ los *animales acuáticos* mueren o se procede a la matanza con fines de control sanitario, o cuando se han sacrificado o procesado para ~~a través del sacrificio y procesamiento de así como también *animales acuáticos* sacrificados y sus partes, que se destinan al~~ el consumo humano ~~o a~~ u otros fines.

Los desplazamientos de los *productos de los animales acuáticos* y *residuos de animales acuáticos* en, dentro y ~~fuera de~~ desde los *establecimientos de acuicultura* pueden plantear un *riesgo* de transmisión de los *agentes patógenos*. Es el caso particular de una población susceptible que se expone a *productos de animales acuáticos* y a *residuos de animales acuáticos* derivados de *animales acuáticos* infectados, con signos clínicos y subclínicos. ~~Los residuos de alto *riesgo* incluyen *residuos de animales acuáticos* que constituyen, o se sospecha constituyen, un serio *riesgo* sanitario significativo para los *animales acuáticos*.~~ Se deberá evitar en la medida de lo posible la circulación de *residuos de animales acuáticos* en los *establecimientos de acuicultura*. Los residuos se deberán almacenar, transportar, eliminar y tratar siguiendo las orientaciones del Capítulo 4.7. Manipulación, eliminación y tratamiento de residuos de animales acuáticos.

Para los desplazamientos intencionales de *productos de animales acuáticos* y *residuos de animales acuáticos*, se deberá evaluar la posibilidad de presencia de *agentes patógenos* en los *animales acuáticos* de los que se derivan los *productos de animales acuáticos* y los residuos teniendo en cuenta las especies, la fuente y el estatus sanitario.

El *riesgo* de transmisión de los *agentes patógenos* a través de los *productos de animales acuáticos* y *residuos de animales acuáticos* ~~se puede~~ se deberá evaluar y gestionar tomando en cuenta las siguientes~~; las posibles~~ medidas de mitigación ~~se realizarán~~:

a) determinar el *riesgo* de *enfermedad* potencial de los productos y *residuos de animales acuáticos* para los *animales acuáticos* en el establecimiento y el entorno;

b) ~~aislando~~ gestionar los *productos de animales acuáticos* y los *residuos de animales acuáticos* dentro del *establecimiento de acuicultura* en áreas que se encuentran aisladas ~~las áreas donde se manipulan *productos de animales acuáticos* y residuos provenientes~~ de las poblaciones de *animales acuáticos* para minimizar los *riesgos* de transmisión de *enfermedades* identificados;

c) garantizar que se han implementado procedimientos ~~sistemas~~ adecuados para la colecta, tratamiento (inactivación de los *agentes patógenos*), transporte, almacenamiento o eliminación de *productos de animales acuáticos* y de *residuos de animales acuáticos* con el fin de minimizar los *riesgos* identificados de transmisión de enfermedad ~~de los *agentes patógenos*~~.

3. Agua

El agua es un recurso importante que favorece la productividad y la sanidad de los *animales acuáticos*, pero que puede presentar un *riesgo* de introducción en, de propagación dentro y de liberación de *agentes patógenos* desde los *establecimientos de acuicultura*. Se deberá identificar y tomar en consideración la fuente de aprovisionamiento del agua y cómo puede representar un vínculo epidemiológico entre el *establecimiento de acuicultura* y otras poblaciones silvestres o de cría o plantas de procesamiento. Se deberá estudiar la exposición al agua del transporte y al agua de lastre.

El *riesgo* de que el *establecimiento de acuicultura* esté expuesto a agua que contenga *agentes patógenos* puede estar influenciado por la categoría del sistema de producción acuícola, sabiendo que la probabilidad es más alta para los sistemas semi-abiertos que para los cerrados. Toda agua que provenga de *animales acuáticos* con un estatus sanitario más bajo o desconocido presenta un *riesgo* potencial de transmisión de *agentes patógenos* a *animales acuáticos* con un estatus sanitario más alto.

El *riesgo* de transmisión de *agentes patógenos* a través del agua se ~~puede~~ deberá evaluar y gestionar tomando en cuenta las siguientes medidas de mitigación ~~de distintas formas~~:

a) Cuando sea posible, elegir fuentes de agua que estén totalmente libres de poblaciones de *animales acuáticos* susceptibles y de *agentes patógenos* de preocupación. Tales fuentes de agua pueden incluir aguas subterráneas dulces o salinas, agua de la red municipal sin cloro y agua de mar artificial. Estas fuentes de agua pueden ser particularmente aptas para los *animales acuáticos* con un alto estatus sanitario, tales como las especies reproductoras.

b) Asegurar un nivel apropiado de cribado, filtrado o *desinfección* (de conformidad con el Capítulo 4.3.) de las aguas provenientes de fuentes que pueden contener *especies susceptibles* y presentar un *riesgo* de transmisión de *agentes patógenos* (por ejemplo, océanos, arroyos o lagos). El tipo y el nivel de tratamiento requerido dependerá de los *riesgos* identificados.

c) Proveer un nivel apropiado de filtrado~~,~~ y *desinfección* ~~o contención~~ (de conformidad con el Capítulo 4.3.) de las aguas efluentes (y de los residuos filtrados asociados) de los *establecimientos de acuicultura* (o mataderos asociados o instalaciones de procesamiento) en donde pueda estar presente un *riesgo* de transmisión de *agentes patógenos* a *animales acuáticos* silvestres o a otros *establecimientos de acuicultura* con *especies susceptibles*. El tipo y el nivel de tratamiento requerido dependerá de los *riesgos* identificados.

d~~c~~) Garantizar que la posición de las tomas de entrada y salida de agua para los *establecimientos de acuicultura* con sistemas semi-cerrados y cerrados, al igual que la ubicación de los *establecimientos de acuicultura* semi-abiertos, minimice la contaminación proveniente de otras poblaciones de cría o *silvestres* o plantas de procesamiento, teniendo en cuenta factores tales como la distancia y las corrientes de agua.

e) Evaluar la probabilidad de ingreso de agua contaminada a través del desbordamiento de fuentes externas o de una infraestructura defectuosa (por ejemplo, tuberías con fugas, desagües bloqueados, muros de contención con grietas) y aplicar las medidas apropiadas a nivel de la gestión o de las infraestructuras.

f) Evaluar los *riesgos* y los procedimientos establecidos para tratar y eliminar los residuos de agua que resultan del transporte de *animales acuáticos*.

4. Alimento para animales (piensos)

Los *alimentos para animales* pueden ser una importante ruta de transmisión de los *agentes patógenos* a los *animales acuáticos*. Los *piensos* pueden estar infectados inicialmente con *agentes patógenos* o contaminarse durante la colecta, el transporte, el almacenamiento y el procesamiento de las materias primas utilizadas como *ingredientes de los alimentos para animales*. Una higiene baja puede contribuir a la contaminación durante la fabricación, el transporte, el almacenamiento o el uso de los *alimentos para animales*.

En los sistemas de producción cerrados o semi-cerrados puede existir un alto nivel de control de los *piensos* para los *animales acuáticos*. Sin embargo, en los sistemas de producción semi-abiertos, los *animales acuáticos* pueden obtener alimento de su entorno (por ejemplo, moluscos que atraviesan los filtros o depredación de peces silvestres por peces de cultivo ~~que pueden quedar atrapados~~ ~~predadores~~ en las redes de las jaulas).

El *riesgo* de transmisión de *agentes patógenos* a través de los *alimentos para animales* se ~~puede~~ deberá evaluar y gestionar mediante las medidas de mitigación ~~como se describe~~ consignadas en el Capítulo 4.8., por ejemplo, utilizando *piensos* e *ingredientes de piensos* que:

a) se hayan sometido a un procesamiento suficiente para inactivar los *agentes patógenos* de preocupación;

b) provengan de fuentes declaradas libres de los *agentes patógenos* de preocupación o se haya confirmado (por ejemplo, mediante pruebas) que los *agentes patógenos* no están presentes en los *piensos* e *ingredientes de piensos* ~~la mercancía;~~

c) hayan sido procesados, fabricados, almacenados, ~~y~~ transportados y distribuidos durante la alimentación de los *animales acuáticos* de manera que se prevenga toda contaminación por *agentes patógenos*.

5. Fómites

Los equipos, *vehículos,* material de embalaje, prendas, calzado, sedimentos, infraestructura y otros fómites pueden transferir mecánicamente *agentes patógenos* en, dentro y desde un *establecimiento de acuicultura*.

~~El nivel de *riesgo*~~ La posibilidad de transferencia de *agentes patógenos* dependerá de la estabilidad del *agente patógeno* en el ambiente, de la presencia y la naturaleza de la materia orgánica en la superficie de los fómites, así como también del tipo de superficie y de su capacidad para retener el agua. ~~El *riesgo*~~ La probabilidad de transferencia de *agentes patógenos* es mayor en los objetos contaminados que son difíciles de limpiar y desinfectar. Los equipos que se comparten entre los *establecimientos de acuicultura*, entre *establecimientos de acuicultura* e instalaciones de procesamiento o entre las distintas unidades de producción con un estatus sanitario diferente dentro de un *establecimiento de acuicultura* pueden acarrear la propagación de *agentes patógenos* ~~presentar un *riesgo* más alto, comparado con equipos nuevos o especializados. *El riesgo* La probabilidad~~ El *riesgo* de transmisión de *agentes patógenos* a través de fómites ~~se puede~~ se deberá evaluar y gestionar~~; las posibles~~ tomando en cuenta las siguientes medidas de mitigación ~~se realizarán~~:

a) Evaluar el *riesgo* de *enfermedad* asociado con ~~a~~ todos los fómites que se ~~introducen~~ mueven en, dentro o desde el *establecimiento de acuicultura.* ~~en función del *riesgo* de *enfermedad* que representan;~~

b) Garantizar que se dispone de procedimientos y de la infraestructura para limpiar y desinfectar los fómites, incluyendo en las áreas consagradas a la carga y entrega, antes de la entrada en los *establecimientos de acuicultura*. Las recomendaciones para la limpieza y *desinfección* de fómites se describen en el Capítulo 4.3.

~~*c)* asignando equipos exclusivos para usar en las unidades de producción de distinto estatus sanitario. Cuando los equipos se utilizan en múltiples unidades de producción, deberán limpiarse y desinfectarse antes de su trasladado entre las unidades.~~

c) Siempre que sea posible, asignar artículos que son difíciles de desinfectar o que tienen una alta probabilidad de contaminación a un *establecimiento de acuicultura* específico, en lugar de desplazarlos entre *establecimientos de acuicultura* después de la *desinfección*.

d) Aplicar las medidas de mitigación descritas en los apartados *a)* a *c)* anteriores a los desplazamientos de fómites entre unidades de producción dentro de un *establecimiento de acuicultura*, con medidas determinadas basadas en una evaluación del ~~los~~ *riesgo~~s~~* de transmisión de *enfermedad*.

6. Vectores

Los *vectores* pueden transportar *agentes patógenos* a *animales acuáticos* susceptibles en los *establecimientos de acuicultura*. ~~Esto i~~Incluyen los *animales acuáticos* silvestres que ingresan al agua a través del sistema de suministro de agua, los predadores, las aves silvestres, los carroñeros, y las plagas, tales como roedores~~, así como las personas.~~ Los *vectores* también pueden transferir *agentes patógenos* dentro~~, en~~ y desde un *establecimiento de acuicultura*~~, ya sea por transferencia mecánica o como una fase de desarrollo del *agente patógeno* dentro del *vector*~~. ~~El *riesgo* de una exposición no intencional a los *vectores* se verá influenciada por la categoría del sistema de producción de *acuicultura*.~~

~~El *riesgo*~~ La probabilidad de transferencia de *agentes patógenos* a través de los *vectores* varía según ~~la especie~~ el tipo del *vector*, la naturaleza del *agente patógeno*, la categoría del sistema de producción acuícola y el nivel de *bioseguridad*. ~~Para mitigar los riesgos asociados a los *vectores*, también se pueden aplican las medidas identificadas para mitigar los *riesgos* asociados con los *animales acuáticos*, descritas en el apartado 1. Las medidas de mitigación para otros *vectores* incluyen:~~

El *riesgo* de transmisión de *agentes patógenos* a través de los *vectores* se deberá evaluar y gestionar tomando en cuenta las siguientes medidas de mitigación:

a) ~~redes de malla (para prevenir el acceso de las aves);~~ Utilización de medidas físicas de mitigación con el fin de prevenir el acceso de *vectores* en los *establecimientos de acuicultura*, entre ellas:

i) filtrar o tamizar el agua que entra y sale en los sistemas de producción de *acuicultura* semi-cerrados y cerrados para prevenir la entrada de *animales acuáticos* silvestres;

ii) instalar alrededor de los sistemas de producción de *acuicultura* en tierra una cerca o muro para prevenir la entrada de animales y personas, con una barrera para un acceso controlado;

iii) instalar alrededor de los sistemas de producción flotantes barreras en el perímetro del establecimiento con el fin de prevenir el contacto con *animales acuáticos* silvestres y otros animales o su introducción;

iv) cubrir con redes los sistemas abiertos de producción acuícola para prevenir el acceso de aves.

~~*b)* barreras en el perímetro del establecimiento para prevenir la entrada de otros animales (por ejemplo, cercos eléctricos);~~

~~*b)* Control del acceso de personal a los *establecimientos de acuicultura* a través de la creación de un límite definido entre el área de riesgo exterior y el área de bioseguridad interior, que abarca las instalaciones que sirven para:~~

~~*i)* el cambio de ropa o de calzado o uso de cubiertas desechables (capuchas, capas y recubrimientos de calzado);~~

~~*ii)* la *desinfección* de manos y el uso de pediluvios para la desinfección del calzado.~~

bc) ~~c~~Control de pestes ~~y almacenamiento seguro de alimentos y de los animales muertos~~.

7. Personal y visitantes

a) Controlar el acceso de personal y visitantes a los *establecimientos de acuicultura* a través de la creación de un límite definido entre el área de riesgo exterior y el área de *bioseguridad* interior, que abarque las instalaciones que sirven para:

i) el cambio de ropa o de calzado o uso de prendas desechables (cofias, capas y cubrecalzado);

ii) la *desinfección* de manos y el uso de pediluvios ~~para la desinfección del calzado~~.

b) Informar a todos los visitantes y supervisar que cumplan con el *plan de bioseguridad*.

Artículo 4.X.7.

Análisis del riesgo

El *análisis del riesgo* constituye un enfoque aceptado para evaluar las amenazas de *bioseguridad* y que se emplea para respaldar el desarrollo de las medidas de mitigación. Un *análisis del riesgo* formal cuenta con cuatro componentes: la *identificación de los peligros*, la *evaluación del riesgo*, la *gestión del riesgo* y la *comunicación sobre el riesgo* ~~(ref. Capítulo 2.1.)~~. Este artículo toma en cuenta los principios del Capítulo 2.1. y los aplica para el desarrollo de los *planes de bioseguridad* en los *establecimientos de acuicultura*.

Un *plan de bioseguridad* puede no requerir necesariamente un *análisis del riesgo* exhaustivo a efectos de evaluar los *riesgos* de *enfermedad* vinculados con las rutas de transmisión. El enfoque elegido dependerá de los objetivos del *plan de bioseguridad* y del nivel de *bioseguridad* que es apropiado para los requisitos de producción específicos del *establecimiento de acuicultura*, la complejidad de las amenazas que hay que tratar, y la disponibilidad de la información y los recursos. En función de estas circunstancias, puede ser apropiado un análisis parcial desarrollado a partir de experiencias pasadas para identificar los *peligros* asociados a las rutas de transmisión pertinentes.

Las tres etapas formales del proceso de *análisis del riesgo* que sustentan un ~~el~~ *plan de bioseguridad* son:

Etapa 1 – Identificación del peligro

La identificación del *peligro* determina cuáles son los *agentes patógenos* que deben someterse a la *evaluación del riesgo*. Un *peligro* puede incluir un *agente patógeno* específico o estar definido en términos más generales como un grupo de *agentes patógenos*. Esta etapa incluye la identificación y la colecta de la información pertinente sobre los *agentes patógenos* que pueden causar *enfermedades* en las poblaciones de *animales acuáticos* dentro de un *establecimiento de acuicultura*. Este proceso deberá considerar el *estatus sanitario* del establecimiento y, para los sistemas de producción semi-abiertos y semi-cerrados, el *estatus sanitario* de los entornos epidemiológicamente vinculados. ~~La segunda etapa consiste en identificar las *enfermedades* conocidas y las *emergentes*, no presentes en el *establecimiento de acuicultura*, que pueden impactar negativamente la población de cría.~~ Se deberán identificar las *enfermedades* conocidas y las emergentes que pueden impactar de manera negativa la población de cría, independientemente de si están presentes en el *establecimiento de acuicultura*.

Para completar las siguientes etapas de la *evaluación del riesgo*, se requiere información sobre los *peligros* identificados que incluye: i) la frecuencia de la aparición, ii) las características biofísicas, iii) la probabilidad de detección si existe, iv) las posibles rutas de transmisión (detalladas en el Artículo 4.X.6.). Muchos de los *peligros* pueden compartir la misma ruta. ~~Un *peligro* puede incluir un *agente patógeno* específico o estar definido en términos más generales como un grupo de *agentes patógenos*~~.

Etapa 2 – Evaluación del riesgo

Una *evaluación del riesgo* se puede iniciar una vez que se haya identificado que existe un *peligro* ~~biológico~~ y se haya reunido la información requerida en la etapa 1. El objetivo de la *evaluación del riesgo* es establecer una estimación del *riesgo*, que es el resultado de la probabilidad y de las consecuencias de la introducción de~~l~~ un *agente patógeno*, de la propagación dentro o de la liberación desde el *establecimiento de acuicultura*.

Una *evaluación del riesgo* puede ser cuantitativa o cualitativa. Ambos métodos requieren el mismo modelo conceptual que identifique las etapas necesarias de la introducción, el establecimiento y la propagación del *peligro*. En una evaluación cualitativa, la introducción y el establecimiento se estiman utilizando descriptores de probabilidad. Una evaluación cuantitativa requiere datos que permitan estimar la probabilidad. En la mayoría de las circunstancias, ~~es posible~~ se evaluarán cualitativamente la posibilidad ~~las rutas~~ de transmisión de *enfermedad* y las consecuencias asociadas, aunque dentro de un marco formal de *evaluación del riesgo*. En los Cuadros 1 y 2 figuran ejemplos de descriptores cualitativos para estimar la probabilidad y las consecuencias. El Cuadro 3 ilustra cómo las estimaciones de la probabilidad y las consecuencias se pueden combinar en una matriz para obtener una estimación del *riesgo*.

Cuadro 1. Descriptores cualitativos de probabilidad

Estimación	**Descriptor**
Remota	~~Nunca se ha escuchado,~~ Muy improbable, pero no es imposible.
Improbable	Puede ocurrir aquí, pero solo en circunstancias excepcionales.
Posible	Pruebas claras sugieren que es posible en esta situación.
Probable	Es probable que ocurra, pero no es seguro.
Segura	Es seguro que ocurra.

Cuadro 2. Descriptores cualitativos de las consecuencias

Estimación	**Descriptor de las consecuencias a nivel del establecimiento de acuicultura**
Insignificante	Impacto no detectable o mínimo. Sin impacto en el comercio.
Menor	~~Impacto~~ Disminución limitada~~o~~ de la producción ~~en la productividad de los *establecimientos de acuicultura* para~~ que sólo afecta un pequeño número de ~~algunas~~ unidades ~~de producción~~ y/o una interrupción del comercio muy limitada y transitoria y/o sólo a corto plazo.
Moderada	~~Amplio impacto sobre la productividad de los *establecimientos de acuicultura* debido al aumento de la mortalidad o la disminución del rendimiento.~~ Disminución de la producción (por ejemplo, aumento sostenido de la mortalidad o tasa de crecimiento en disminución), y/o interrupción del comercio a corto y mediano plazo, lo que resulta en pérdidas financieras.
Mayor	~~Impacto c~~Considerable, disminución de la producción, ~~de los *establecimientos de acuicultura*~~ y/o interrupción del comercio a mediano y a largo plazo, lo que resulta en pérdidas financieras significativas. ~~que acarrea serias limitaciones en el aprovisionamiento e impacto financiero.~~
Catastrófica	~~Despoblación~~ Pérdida completa de la producción ~~del *establecimiento de acuicultura*~~ y posibles barreras para la reanudación de la producción, y/o completa interrupción del comercio, lo que resulta en pérdidas financieras extremas.

Cuadro 3. Matriz para ~~evaluar~~ estimar el riesgo

	Índice de consecuencia					
Estimación de la posibilidad		Insignificante	Menor	Moderado	Mayor	Catastrófico
	Remota	Insignificante	Bajo	Bajo	Bajo	Medio
	Improbable	Bajo	Bajo	Medio	Medio	Alto
	Posible	Bajo	Medio	Medio	Alto	Alto
	Probable	Bajo	Medio	Alto	Alto	Extremo
	Segura	~~Medio~~ Bajo	Alto	Alto	Extremo	Extremo

~~Los resultados de la~~ Las *evaluaciones~~ón~~ del riesgo* dan cuenta de los *peligros* ~~biológicos~~ que necesitan tratarse, los puntos de control críticos en las rutas de transmisión que deben ser abordados y las medidas que son probablemente más eficaces para la reducción del *riesgo*.

Cuadro 4. Interpretación de las estimaciones del riesgo

~~Nivel~~ Estimación del riesgo*	**Explicación y respuesta de gestión**
Insignificante	Nivel aceptable de *riesgo*. No se requiere ninguna acción.
Baja	Nivel aceptable de *riesgo*. Se puede requerir un seguimiento permanente.
Media	Nivel inaceptable de *riesgo*. Revisión y refuerzo de las medidas de mitigación del *riesgo*. ~~Se requiere una gestión activa para reducir el nivel de *riesgo*.~~
Alta	Nivel inaceptable de *riesgo*. Identificación e implementación de medidas adicionales de mitigación del *riesgo*. ~~Se requiere una intervención para mitigar el *riesgo*.~~
Extrema	Nivel de *riesgo* inaceptable. Actuación inmediatamente para mitigar el *riesgo*. ~~Se requiere una intervención urgente para mitigar el nivel de *riesgo*.~~

~~*La estimación del nivel de *riesgo* se determinado por mediante la combinación de las estimaciones de la posibilidad y el índice de consecuencias obtenido utilizando la matriz de *riesgo* (Cuadro 3).~~ Las estimaciones de posibilidad y de las consecuencias se combinan utilizando la matriz del riesgo (cuadro 3) con el fin obtener la estimación del *riesgo*.

Etapa 3 – Gestión del riesgo

La *gestión del riesgo* se utiliza para determinar la respuesta de gestión apropiada para el nivel de *riesgo* evaluado, según se describe en el Cuadro 4. El proceso de *evaluación del riesgo* identifica las etapas dentro de las rutas de transmisión necesarias para que un *riesgo* se materialice y, de este modo, permite determinar las medidas de mitigación más eficaces. Muchos de los *peligros* compartirán las mismas rutas y, por lo tanto, las medidas de mitigación resultarán eficaces frente a más de un *peligro*. La información sobre los *peligros* y sus rutas de introducción (etapa 1) deberá combinarse con una ~~la~~ *evaluación del riesgo* ~~de~~ asociada con cada ~~las~~ ruta~~s~~ (etapa 2) con el fin de identificar las medidas de mitigación del *riesgo* más apropiadas y rentables.

El Artículo X.X.6. describe algunas medidas de mitigación posibles, pertinentes para las distintas rutas de transmisión. Las medidas de mitigación más apropiadas para un *establecimiento de acuicultura* específico dependerán ~~de los *riesgos peligros* identificados,~~ de la eficacia y la fiabilidad de la medida de mitigación, de la categoría del sistema de producción acuícola y de su costo.

Tras la implementación del *plan de bioseguridad*, los *peligros* deberán volverse a evaluar con regularidad y se ajustarán las medidas de acuerdo con los cambios en las estimaciones de *riesgo*.

Artículo 4.X.8.

Desarrollo del plan de bioseguridad

La finalidad principal del *plan de bioseguridad* es reducir la probabilidad de introducción de los *agentes patógenos* en un *establecimiento de acuicultura* y, si los agentes patógenos se introducen, reducir el *riesgo* de una propagación mayor dentro o de una liberación desde un *establecimiento de acuicultura*. El plan documentará las rutas de transmisión identificadas y los resultados de cualquier *análisis del riesgo* realizado (*peligros*, estimación del *riesgo* y medidas de mitigación), así como la información pertinente sobre la implementación en curso, el seguimiento y la revisión del plan.

1. Desarrollo de un plan de bioseguridad

El proceso para desarrollar un *plan de bioseguridad* variará dependiendo de sus objetivos, del nivel de *bioseguridad* apropiado según los requisitos específicos de producción, de la complejidad de las amenazas que se deben tratar y de la disponibilidad de las informaciones y los recursos. Se recomienda tomar en consideración y documentar los siguientes temas:

a) objetivos, campo de aplicación y requisitos reglamentarios del *plan de bioseguridad*;

b) información acerca de los *establecimientos de acuicultura* incluyendo un plan actualizado de la disposición de los edificios y de las unidades de producción (incorporando las *unidades epidemiológicas*, si las hay, y las estructuras y procesos para mantener la ~~los métodos de~~ separación de las mismas), las áreas de carga y descarga, desembalaje, procesamiento, almacenamiento de los *piensos*, depósito de los *residuos de animales acuáticos*, ~~y~~ áreas de recepción, puntos de acceso, así como esquemas de los principales desplazamientos de los *animales acuáticos,* de los *productos de animales acuáticos*, de los residuos de *animales acuáticos*, del agua, los *piensos* y los fómites ~~(incluidos el personal, los equipos y los *vehículos*)~~;

c) las posibles rutas de entrada de los *agentes patógenos* en un *establecimiento de acuicultura*, de propagación o de liberación desde un *establecimiento de acuicultura* (ver Artículo X.X.6. más arriba);

d) un *análisis del riesgo*, incluyendo la identificación de los principales *peligros* de *enfermedad* para el *establecimiento de acuicultura* (ver Artículo X.X.7. más arriba);

e) las medidas de mitigación que se han determinado para tratar los *riesgos* ~~identificados~~;

f) los procedimientos de emergencia en caso de una falla de *bioseguridad*. Entre ellos, se pueden incluir los requisitos de notificación~~,~~ y las medidas de emergencia para erradicar los *agentes patógenos* tales como la despoblación y la eliminación de *animales acuáticos* y la *desinfección* del sitio, de conformidad con los Capítulos 4.3. y 7.4.;

~~*g)* los procedimientos operativos estándar requeridos para acompañar la implementación de las medidas de mitigación, los procedimientos de emergencia y los requisitos de formación del personal;~~

g~~h~~) los procedimientos de comunicación externos e internos, ~~y~~ las funciones y responsabilidades del personal del *establecimiento de acuicultura*, así como la información de contacto básica, por ejemplo, del personal, el *veterinario* de la granja y la *autoridad competente*;

h~~i~~) el calendario de seguimientos y auditorías;

i~~j~~) la evaluación de rendimiento~~.~~;

j) los procedimientos operativos estándar requeridos para respaldar la ~~toda~~ implementación de las medidas de mitigación descritas en el *plan de bioseguridad*, los procedimientos de emergencia y los requisitos de formación del personal del establecimiento.

2. Componentes clave de un plan de bioseguridad

a) Procedimientos operativos estándar (POE)

Los POE describen los procesos de gestión de rutina que deben efectuarse para favorecer la eficacia del *plan de bioseguridad*. Cada POE deberá describir su objetivo, las responsabilidades de los operarios, el procedimiento en sí (incluyendo el mantenimiento de registros), las precauciones y una fecha de revisión.

Se deberá capacitar al personal en la implementación de los POE, lo que incluye completar formularios, listas de verificación y otros registros asociados con cada procedimiento, así como los requisitos de comunicación de rutina.

b) Documentación y registros

El *plan de bioseguridad* describe la documentación necesaria para brindar las pruebas del cumplimiento del plan ~~las medidas de mitigación~~. El nivel de detalle requerido en la documentación dependerá de los resultados de la evaluación de la ruta de transmisión.

Ejemplos de la documentación requerida incluyen ~~pueden incluir~~: la disposición de los *establecimientos de acuicultura*, los desplazamientos de los *animales acuáticos*; ~~salidas de emergencia;~~ el origen y destino al igual que el estatus sanitario de los *animales acuáticos* introducidos en el *establecimiento de acuicultura*, las medidas de cuarentena, los registros de los visitantes del establecimiento, los animales que se han escapado, la densidad de población, las tasas de crecimiento y alimentación, los registros sobre la formación del personal; los tratamientos y la vacunación, la calidad del agua, los eventos de limpieza y *desinfección*, la morbilidad y mortalidad (incluyendo el retiro y la eliminación de los animales muertos), los registros de *vigilancia* y de laboratorio.

c) Procedimientos de emergencia

Deberán desarrollarse e implementarse procedimientos destinados a minimizar el impacto de las emergencias, los eventos de *enfermedad* o la mortalidad inexplicada en los *animales acuáticos*. Estos procedimientos deberán incorporar umbrales claramente definidos que ayuden a identificar un incidente de emergencia y a activar los protocolos de respuesta, incluyendo los requisitos de notificación.

d) Seguimiento sanitario

El seguimiento sanitario como parte del *plan de bioseguridad* implica el seguimiento del *estatus sanitario* de los *animales acuáticos* en los *establecimientos de acuicultura*. Las actividades pueden incluir la *vigilancia* de la *enfermedad*, los controles de rutina de las existencias para los parámetros importantes de sanidad y producción (por ejemplo, por el productor, un *profesional de sanidad de los animales acuáticos* o un *veterinario*), el registro de signos clínicos de *enfermedad*, morbilidad y mortalidad y el análisis de dichos datos (por ejemplo, cálculo de las tasas de morbilidad y ~~la~~ mortalidad ~~y enfermedades~~).

e) Revisión de rutina y auditoría

El *plan de bioseguridad* deberá describir un organigrama de auditoría sistemática encaminado a verificar la implementación y el cumplimiento de los requisitos del *plan de bioseguridad*. La revisión de rutina del *plan de bioseguridad* es necesaria para garantizar que continúa encarando con eficacia los *riesgos* de *bioseguridad*.

El *plan de bioseguridad* también deberá revisarse por lo menos anualmente o como respuesta a los cambios operativos en el *establecimiento de acuicultura*, los cambios en el diseño de las instalaciones, los cambios en los enfoques de cría, la identificación de un nuevo *riesgo* de *enfermedad* o tras un incidente de *bioseguridad* ~~por lo menos anualmente~~. Los incidentes de *bioseguridad*, y las acciones tomadas para remediarlos, deberán documentarse para permitir una reevaluación de los procedimientos operativos estándar.

CAPÍTULO 1.3.

ENFERMEDADES DE LA LISTA DE LA OIE

[…]

Artículo 1.3.3.

Están incluidas en la lista de la OIE las siguientes *enfermedades* de los crustáceos:

– Enfermedad de la necrosis hepatopancreática aguda

– Infección por *Aphanomyces astaci* (plaga del cangrejo de río)

– Infección por *Hepatobacter penaei* (hepatopancreatitis necrotizante)

– Infección por el virus de la necrosis hipodérmica y hematopoyética infecciosa

– Infección por el virus de la mionecrosis infecciosa

– Infección por el nodavirus *Macrobrachium rosenbergii* (enfermedad de la cola blanca)

– Infección por el virus del síndrome de Taura

– Infección por el virus del síndrome de las manchas blancas

– Infección por el virus de la cabeza amarilla genotipo 1

– ~~del hemocito del camarón.~~ Infección por el virus ~~1~~ iridiscente de los decápodos tipo 1.

[…]

Modelo de Artículo 10.X.13. para los capítulos 10.5.; 10.6. y 10.10 específicos de enfermedades de los peces (y o el Artículo 10.4.17. del Capítulo 10.4. para la iInfección por el virus de la anemia infecciosa del salmón)

[…]

Artículo 10.X.13.

Importación, para la acuicultura, de huevos desinfectados de un país, una zona o un compartimento no declarados libres de infección por X

1) Cuando se importen, para la *acuicultura*, huevos desinfectados de una de las especies mencionadas en el Artículo 10.X.2. de un país, una *zona* o un *compartimento* no declarados libres de infección por [el agente patógeno X], la *autoridad competente* del *país importador* deberá evaluar de conformidad con el Capítulo 4.4. el *riesgo* asociado al menos los siguientes aspectos:

   a) al estado de contaminación por el agente patógeno X; del la probabilidad de que el agua utilizada durante la *desinfección* de los huevos esté contaminada por [el agente patógeno X];

   b) la prevalencia de la infección por [el agente patógeno X] en la reserva de genitores (incluyendo los resultados de las mediante pruebas del líquido ovárico y la lechaza), y

   c) la temperatura y el pH del agua utilizada para la *desinfección*.

2) Si la *autoridad competente* del *país importador* concluye que importación es aceptable, deberá solicitar que se apliquen aplicar las siguientes medidas de mitigación del *riesgo*, entre ellas:

   a) *desinfección* de los huevos deberán desinfectarse antes de la importación, de acuerdo con las recomendaciones formuladas en el Capítulo 4.4. o las especificadas por la *autoridad competente* del *país importador*, y

   b) entre la *desinfección* y la importación, los huevos no deberán entrar en contacto con nada que pueda afectar a su estatus sanitario.

   La *autoridad competente* podrá deberá contemplar medidas internas, como una nueva *desinfección* adicional de los huevos a su llegada al *país importador*.

3) Cuando se importen, para la *acuicultura*, huevos desinfectados de una de las especies mencionadas en el Artículo 10.X.2. de un país, una *zona* o un *compartimento* no declarados libres de la infección por [el agente patógeno X], la *autoridad competente* del *país importador* deberá exigir la presentación de un *certificado sanitario internacional aplicable a los animales acuáticos* extendido por la *autoridad competente* del *país exportador* que acredite el cumplimiento de los procedimientos descritos en el apartado 2 *a)* y *b)* del presente artículo.

[…]

CAPÍTULO 10.9.

INFECCION POR EL VIRUS DE LA VIREMIA PRIMAVERAL DE LA CARPA

[…]

Artículo 10.9.2.

Ámbito de aplicación

Las recomendaciones de este capítulo se aplican a las siguientes especies que cumplen con los criterios de inclusión en la lista de especies susceptibles de acuerdo con el Capítulo 1.5.:

~~todas las variedades y subespecies de la carpa común (*Cyprinus carpio*), carpa cabezona *(Aristichthys nobilis)*, brema *(Abramis brama)*, pescado blanco del Caspio *(Rutilus kutum)*, *Pimephales promelas*, *Notemigonus crysoleucas*, carpa dorada *(Carassius auratus)*, carpa herbívora *(Ctenopharyngodon idella)*, rutilo *(Rutilus rutilus)* y siluro *(Silurus glanis)*.~~

Familia	**Nombre científico**	**Nombre común**
Cyprinidae	*Abramis brama*	Brema
	Aristichthys nobilis	Carpa cabezona
	Carassius auratus	Carpa dorada
	Ctenopharyngodon idella	Carpa herbívora
	Cyprinus carpio	Carpa común (todas las variedades y subespecies)
	Danio rerio	Pez zebra
	Notemigonus crysoleucas	Carpita dorada
	Pimephales promelas	Piscardo
	Rutilus kutum	Pescado blanco del Caspio
	Rutilus rutilus	Rutilo
Siluridae	*Silurus glanis*	Siluro

[…]

CAPÍTULO 10.10.

INFECCIÓN POR EL VIRUS DE LA SEPTICEMIA HEMORRÁGICA VIRAL

[...]

Artículo 10.10.2.

Ámbito de aplicación

Las recomendaciones de este capítulo se aplican a las siguientes especies que cumplen con los criterios para la inclusión como susceptibles de conformidad con el Capítulo 1.5.: ~~trucha arco iris *(Oncorhynchus mykiss)*, reo *(Salmo trutta)*, tímalo *(Thymallus thymallus)*, coregonos (*Coregonus* sp.), lucio *(Esox lucius)*, rodaballo *(Scophthalmus maximus)*, arenque y espadín (*Clupea* sp.), salmón del Pacífico (*Oncorhynchus* sp.), bacalao *(Gadus morhua)*, bacalao del Pacífico *(Gadus macrocephalus)*, *Gadus aeglefinus* y *Onos mustelus*. Estas recomendaciones se aplican también a todas las demás *especies susceptibles* mencionadas en el *Manual Acuático* que sean objeto de *comercio internacional.*~~

Familia	**Nombre científico**	**Nombre común**	~~**Genotipo**~~
Ammodytidae	*Ammodytes hexapterus*		~~IVa~~
Aralichthyidae	*Paralichthys olivaceus*	Falso halibut del Japón	~~IVa~~
Carrangidae	*Trachurus mediterraneus*	Jurel mediterráneo o jurel blanco	~~Ie~~
Centrarchidae	*Ambloplites rupestris*	Perca de roca	~~IVb~~
	Lepomis gibbosus	Perca sol	~~IVb~~
	Lepomis macrochirus	Mojarra oreja azul	~~IV, IVb~~
	Micropterus dolomieu	Lobina de boca pequeña	~~IVb~~
	Micropterus salmoides	Perca atruchada	~~IVb~~
	Pomoxis nigromaculatus	Perca plateada	~~IVb~~
Clupeidae	*Alosa immaculata*		~~Ie~~
	Sardina pilchardus	Sardina europea o sardina común	
	Clupea harengus	Arenque del Atlántico	~~Ib, III~~
	Clupea pallasii pallasii	Arenque del Pacífico	~~Iva~~
	Dorosoma cepedianum	Sábalo molleja	~~IVb~~
	Sardinops sagax	Sardina	~~IVa~~
	Sprattus sprattus	Espadín	~~Ib~~
Cyclopteridae	*Cyclopterus lumpus*	Lumpo o ciclóptero	~~IVd~~
Cyprinidae	*Danio rerio*	Pez cebra	~~IVa~~
	Notropis hudsonius	Pajarito cabezidura	~~IVb~~
	Notropis atherinoides		~~IVb~~
	Pimephales notatus		~~IVb~~
	Pimephales promelas	Piscardo	~~IVb~~

Anexo 6 (cont.)

Familia	**Nombre científico**	**Nombre común**	**~~Genotipo~~**
Embiotocidae	*Cymatogaster aggregata*	Mojarra brillosa	~~IVa~~
Engraulidae	Engraulis encrasicolus	Boquerón	~~Ic~~
Esocidae	*Esox lucius*	Lucio	~~IVb~~
	Esox masquinongy		~~IVb~~
Fundulidae	*Fundulus heteroclitus*		~~IVc~~
Gadidae	*Gadus macrocephalus*	Bacalao del Pacífico	~~IVa~~
	Gadus morhua	Bacalao	~~Ib, III~~
	Merlangius merlangus	Plegonero	~~Ic~~
	Micromesistius poutassou	Bacaladilla	~~Ib, III~~
	Trisopterus esmarkii	Faneca noruega	~~Ib, III~~
Gasterosteidae	*Gasterosteus aculeatus*	Espinosillo	~~IVc~~
Gobiidae	*Neogobius melanostomus*	Gobio redondo	~~IVb~~
	Pomatoschistus minutus	Gobio de arena	~~Ib~~
Ictaluridae	~~*Ictalurus*~~ *Ameiurus nebulosus*	Bagre pardo	~~IVb~~
Labridae	*Centrolabrus exoletus*	Centrolabro	~~III~~
	Ctenolabrus rupestris	Tabernero	~~III~~
	Labrus bergylta	Maragota	~~III~~
	Labrus mixtus	Gallano	~~III~~
	Symphodus melops	Porredana	~~III~~
Lotidae	*Gaidropsarus vulgaris*	Mollareta	~~Ic~~
Moronidae	*Morone americana*	Lubina blanca	~~IVb~~
	Morone chrysops	Robalo blanco /perca blanca	~~IVb~~
	Morone saxatilis	Lubina estriada	~~IVb, IVc~~
Mullidae	*Mullus barbatus*	Salmonete de fango	~~Ic~~
Osmeridae	*Thaleichthys pacificus*	Eulacón	~~IVa~~
Percidae	*Sander vitreus*	Lucioperca americana	~~IVb~~
	Perca flavescens	Perca canadiense	~~IVb~~
Petromyzontidae	*Lampetra fluviatilis*	Lamprea de río	~~II~~
Pleuronectidae	*Limanda limanda*	Lenguadina	~~Ib~~
	Platichthys flesus	Platija	~~Ib~~
	Pleuronectes platessus	Solla	~~III~~
Rajidae	*Raja clavata*	Raya de clavos	~~Ic~~

Familia	**Nombre científico**	**Nombre común**	~~**Genotipo**~~
Salmonidae	*Coregonus artedii*	Oregono de artedi	~~IVb~~
	Coregonus clupeaformis	Coregono del lago	~~IVb~~
	Coregonus lavaretus	Lavareto	~~Ia~~
	Oncorhynchus kisutch	Salmón plateado	~~IVa~~
	Oncorhynchus mykiss	Trucha arco iris	~~Ia-e, III, IVb~~
	Oncorhynchus mykiss X *Oncorhynchus kisutch* hybrids	Trucha arco iris X Híbridos del coho	~~Ia~~
	Oncorhynchus tshawytscha	Salmón real	~~IVa, IVb~~
	Salmo marmoratus	Trucha marmorata	~~Ia~~
	Salmo salar	Salmón del Atlántico	~~Ia, Ib, II, III, IVa~~
	Salmo trutta	Trucha marina	~~Ia, Ib~~
	Salvelinus namaycush	Trucha lacustre	~~Ia, IVa, IVb~~
	Thymallus thymallus	Tímalo	~~I~~
Scophthalmidae	*Scophthalmus maximus*	Rodaballo	~~Ib, III~~
Sciaenidae	*Aplodinotus grunniens*	Roncador de agua dulce	~~IVb~~
Scombridae	*Scomber japonicus*	Estornino del Pacífico	~~IVa~~
Soleidae	*Solea senegalensis*	Lenguado senegalés	~~III~~
Uranoscopidae	*Uranoscopus scaber*	Rata	~~Ie~~

[…]

GLOSARIO

RESIDUOS DE ANIMALES ACUÁTICOS

designa todo resto ~~todo lo que se genera~~ de ~~los~~ *animal~~es~~ acuático~~s~~* ~~que han muerto, o que han sido sacrificados con fines de control sanitario o sacrificado y procesado para consumo humano o para otros fines. Lo que puede incluir todo el cuerpo de los *animales acuáticos*, algunas de sus partes~~ o líquidos asociados destinados a su eliminación.

PRODUCTOS DE ANIMALES ACUÁTICOS

designa los *animales acuáticos* no viables ~~inviables~~, partes de los *animales acuáticos* o productos manufacturados que contienen cualquier material derivado ~~y los productos~~ de *animales acuáticos* que están destinados a la venta o al comercio.

VECTOR

designa cualquier organismo vivo, que no sean *especies susceptibles*, que transporta un *agente patógeno* a una población de *animal~~es~~ acuático~~s~~* susceptible~~s~~ ~~o a sus alimentos o al entorno inmediato. El *agente patógeno* puede pasar o no por un ciclo de desarrollo dentro del vector.~~

Enmiendas propuestas para remplazar el término "residuos" por "residuos de animales acuáticos" en el *Código Acuático*

Artículo	**Página**	**Cambio propuesto**
Guía del usuario, C. Cuestiones específicas, 7), última frase		La evaluación para la inclusión de estos productos de animales acuáticos en estos artículos se basa en la forma y presentación del producto, el volumen previsto de ~~residuos~~ tejidos de *residuos de animales acuáticos* generados por el consumidor y la presencia probable del agente patógeno en los ~~residuos~~ *residuos de animales acuáticos*.
2.1.4., 2.c), ultimo guion		- métodos de eliminación de los ~~despojos~~ *residuos de animales acuáticos*.
4.2.3., 2.i)		i) la eliminación de ~~residuos de~~ *residuos de animales acuáticos*
4.3.6.	60	Estas condiciones incluyen un alto nivel de *riesgo* de *enfermedad* (debido a la importancia de la *enfermedad*), una importante concentración de agentes patógenos, volúmenes potencialmente altos de *animales acuáticos* infectados y de ~~residuos~~ *residuos de animales acuáticos.*
4.7.1.	71	El objetivo de este capítulo es proporcionar pautas para el almacenamiento, transporte, eliminación y tratamiento de los ~~residuos~~ *residuos de animales acuáticos* a fin de controlar los *riesgos* para la sanidad de los *animales acuáticos*.
4.7.2.	71	Este capítulo se aplica a los ~~residuos de~~ *residuos de animales acuáticos* derivados de: i) operaciones rutinarias de *acuicultura*; ii) transformación en el litoral, independientemente del origen; iii) sacrificio masivo con fines de control sanitario y iv) mortalidad masiva (incluso en el medio natural).
4.7.3.	71	A efectos de este capítulo: ~~Restos de animales acuáticos: designa el cuerpo entero, o partes del mismo, de los *animales acuáticos* hallados muertos, o que hayan sido sacrificados con fines de control sanitario o para el consumo, que no se destinan al consumo humano.~~ Residuos de alto riesgo: designa los ~~residuos de~~ *residuos de animales acuáticos* que entrañan o supuestamente entrañan un *riesgo* grave para la salud de los *animales acuáticos* o del hombre. Residuos de bajo riesgo: designa los ~~residuos de~~ *residuos de animales acuáticos* no considerados de alto riesgo.
4.7.4.	71	La autoridad competente deberá supervisar la eliminación eficiente y eficaz de los residuos de residuos de animales acuáticos. 1) acceso del personal pertinente a las instalaciones físicas, a la logística y a los datos, en cooperación con las partes interesadas, incluido el acceso de la autoridad competente a los ~~residuos de~~ *residuos de animales acuáticos*; 2) control de los desplazamientos de animales y autoridad para hacer excepciones en ciertas condiciones de *bioseguridad* (por ejemplo, para transportar ~~residuos de~~ *residuos de animales acuáticos* al lugar donde van a ser eliminados);

4.7.5.	72	Una vez recolectados, los ~~residuos de~~ *residuos de animales acuáticos* deberán almacenarse el tiempo mínimo posible; no obstante, si el almacenamiento es necesario, deberá disponerse de capacidad suficiente para la cantidad prevista de ~~residuos de~~ *residuos de animales acuáticos* y la *autoridad competente* podrá exigir otras medidas. […] Los contenedores para almacenar los ~~residuos de~~ *residuos de animales acuáticos* deben ser herméticos y seguros para evitar el contacto con *animales acuáticos*, aves u otros animales y con el personal no autorizado. Los ~~residuos de~~ *residuos de animales acuáticos* infectados por el agente causal, real o presunto, de una *enfermedad* inscrita en el *Código Acuático*, no podrán ser transportados sin autorización de la *autoridad competente*. […] Si los ~~residuos de~~ *residuos de animales acuáticos* de bajo riesgo son contaminados por residuos de alto riesgo, también deberán ser considerados como residuos de alto riesgo. Los contenedores utilizados para transportar ~~residuos de~~ *residuos animales acuáticos* deberán ser herméticos y llevar una etiqueta con la indicación de su contenido. […]
4.7.6.	72	1. Requisito de autorización Todas las plantas de eliminación de ~~residuos de~~ *residuos animales acuáticos* deberán disponer de la autorización de la *autoridad competente*. […] 2. Condiciones de autorización Para obtener la autorización, una planta de eliminación ~~residuos de~~ *residuos* de *animales acuáticos* deberá reunir las siguientes condiciones: […] d) cumplir los requisitos para la manipulación de ~~residuos de~~ *residuos de animales acuáticos* y *productos de animales acuáticos* especificados por la *autoridad competente*. 3. Requisitos de procedimientos […] La planta de eliminación deberá aplicar procedimientos que minimicen el *riesgo* de propagación de *agentes patógenos*, tales como: c) manipulación y tratamiento de los ~~residuos de~~ *residuos de animales acuáticos* sin dilación en cuanto se reciben; […]
4.7.7.	73	1. Transformación industrial de residuos animales […] El proceso implica por lo general precalentamiento a 50–60°C, seguido de cocción de la materia prima de *residuos de animales acuáticos* a 95–100°C durante 15 a 20 minutos. 2. Incineración […] Los incineradores móviles con cortina de aire permiten realizar el proceso in situ sin necesidad de transportar los s ~~residuos de~~ *residuos de animales acuáticos*. Los incineradores sólo tienen capacidad para tratar volúmenes limitados de ~~residuos de~~ *residuos de animales acuáticos*
4.7.7.	74	6. Ensilado […] El ensilado de ~~residuos~~ *residuos* de *animales acuáticos* en un ácido orgánico tal como el ácido fórmico es un método eficaz para inactivar la mayor parte de *agentes patógenos* en 48 horas […]

4.7.7.	74	7. Inhumación […] Siempre que sea posible, los ~~residuos~~ *residuos animales acuáticos* deberán someterse a un tratamiento que garantice la inactivación de los *agentes patógenos* antes de su inhumación. Para seleccionar un lugar de inhumación aceptable, deberán tomarse en consideración los siguientes elementos: b) Acceso – un acceso fácil para los equipos y la entrega de los ~~residuos~~ *residuos animales acuáticos*. Tal vez sea necesario prever el uso de cercas y restringir la entrada al sitio. c) Construcción de fosos […] Las dimensiones del foso dependen del volumen de los ~~residuos~~ *residuos* de *animales acuáticos* que se van a inhumar y de la facilidad de relleno. d) Cierre del foso – el contenido deberá ser cubierto con cal viva (CaO) a una tasa de 85 kg por 1000 kg de ~~residuos~~ *residuos animales acuáticos* para acelerar la descomposición y evitar la búsqueda de sustento en los residuos. 8. Quema en hoguera La hoguera puede no ser adecuada para grandes cantidades de ~~residuos~~ *residuos animales acuáticos*. […] *a)* Acceso – para transportar el material para preparar la hoguera y mantener el fuego, para la entrega de combustible y de los ~~residuos~~ *residuos* de *animales acuáticos*. […]Si la quema se efectúa correctamente, los ~~residuos~~ *residuos de animales acuáticos* son destruidos en 48 horas.
4.7.8.	75	1. Ensilado El ensilado de ~~residuos~~ *residuos de animales acuáticos* en un ácido orgánico tal como el ácido fórmico es un método eficaz para inactivar la mayor parte de *agentes patógenos* en 48 horas.
5.4.2.	93	[…] Los criterios de inclusión de estos productos en el apartado 1 del Artículo X.X.11. (capítulos específicos sobre las *enfermedades* de los moluscos), del Artículo X.X.12. (capítulos sobre las *enfermedades* de los anfibios, crustáceos y peces) y del Artículo 10.4.16. toman en consideración la forma y presentación del producto, el volumen previsto de ~~residuos de~~ tejidos de *residuos de animales acuáticos* generados por el consumidor y la presencia probable de *agente patógeno* en los ~~residuos~~ *residuos de animales acuáticos*. […] Se presupone que (i) los *productos de animales acuáticos* se utilizan para consumo humano exclusivamente, (ii) no siempre será posible manipular los ~~residuos~~ *residuos de animales acuáticos*. de modo apropiado para disminuir la introducción del *agente patógeno*; el nivel del riesgo depende de las prácticas de eliminación de residuos en el país o territorio de cada Miembro, […]

5.4.2.	93	***Criterios*** […] SEA 2) incluir una pequeña cantidad de residuos de tejido crudo ~~residuos~~ de *residuos de animales acuáticos* generados por el consumidor que sea improbable resulte en la introducción y el establecimiento del *agente patógeno*; SEA 3) el *agente patógeno* no se encuentra normalmente en esos ~~residuos~~ *residuos de animales acuáticos* generados por el consumidor.
6.5.3.	129	3. Evaluación del riesgo de introducción […] - datos sobre las tendencias y la aparición de microorganismos resistentes obtenidos mediante la *vigilancia* de los *animales acuáticos*, de los productos de animales acuáticos y de los consiguientes ~~residuos~~ *residuos de animales acuáticos*. 4. Evaluación de la exposición […] - métodos de eliminación de los ~~desechos~~ *residuos de animales acuáticos* y probabilidad de exposición humana a microorganismos resistentes o a determinantes de resistencia a través de dichos residuos; […]

Modelo de artículos para declarar la ausencia de enfermedad

Nota: los periodos de tiempo de estos modelos de artículos los determinará la Comisión para los Animales Acuáticos para cada enfermedad a partir de los criterios que se incluirán en el Capítulo revisado 1.4. Por esta razón, los periodos se muestran con [X] para indicar que el periodo se determinará para cada enfermedad específica. Cuando figura un periodo (por ejemplo, "los [dos] últimos años") esto indica un periodo previsto por defecto que puede variar dependiendo de las circunstancias de cada enfermedad.

Artículo X.X.4.

[Nota: este nuevo artículo destaca los requisitos generales para realizar una autodeclaración de ausencia de enfermedad para un país, zona o compartimento.]

Requisitos para la declaración de ausencia del [PATÓGENO X]

Un País Miembro puede hacer una *autodeclaración de ausencia* de infección por el [PATÓGENO X] para todo el país una *zona* o *compartimento* de conformidad con las disposiciones de los Artículos X.X.5 a X.X.8., según el caso. La autodeclaración debe realizarse de conformidad con los requisitos relevantes del *Código Acuático*, además de que el País Miembro:

1) cumpla las disposiciones del Capítulo 3.1. sobre la Calidad de los *Servicios de sanidad de los animales acuáticos*; y

2) utilice los métodos de diagnóstico apropiados recomendados en el *Manual Acuático*; y

3) reúna todos los requisitos del Capítulo 1.4. que son relevantes para la autodeclaración de ausencia de enfermedad.

Artículo X.X.5.

[Nota: equivalente al Artículo existente X.X.4.]

País libre de infección por el [PATÓGENO X]

Si un país comparte una *zona* con uno o más países, sólo podrá hacer una *autodeclaración de ausencia* de infección por el [PATÓGENO X] si todas las áreas cubiertas por cuerpos de aguas compartidas se encuentran dentro de países o *zonas* que han sido declarados libres de infección por el *[PATÓGENO X]* (véase el Artículo X.X.6.).

Como se describe en el Artículo 1.4.X., un País Miembro podrá hacer una *autodeclaración de ausencia* de infección por el [PATÓGENO X] para todo su *territorio* si:

1) ninguna *especie susceptible* de las mencionadas en el Artículo X.X..2. está presente y se han reunido ininterrumpidamente las *condiciones elementales de bioseguridad* durante, por lo menos, los [dos] últimos años;

O

2) no ha ocurrido ninguna infección por el [PATÓGENO X] durante, por lo menos, los [diez] últimos años, y:

   a) el País Miembro puede demostrar condiciones propicias para la manifestación clínica de la infección por el [PATÓGENO X] , de acuerdo con lo indicado en el capítulo correspondiente del *Manual Acuático*, y

   b) se han reunido ininterrumpidamente las *condiciones elementales de bioseguridad* descritas en el Capítulo 1.4. durante, por lo menos, los [10] últimos años;

O

3) se ha aplicado una *vigilancia específica* en la *zona*, de conformidad con lo descrito en el Capítulo 1.4., durante, por lo menos, los [dos] últimos años y no se ha detectado la presencia del [PATÓGENO X] y:

a) se han reunido ininterrumpidamente las *condiciones elementales de bioseguridad* durante, por lo menos, [un] año antes del inicio de la *vigilancia específica*;

O

4) había hecho previamente una *autodeclaración de ausencia* de infección por el [PATÓGENO X] para la *zona* y perdió posteriormente su estatus libre por haberse detectado el [PATÓGENO X], pero se han dado las condiciones siguientes:

a) en cuanto se detectó el [PATÓGENO X], el área afectada fue declarada *zona infectada* y se estableció una *zona de protección*, y

b) las poblaciones infectadas dentro de la *zona infectada* se han sacrificado y eliminado con medios que reducen al mínimo la probabilidad de una mayor transmisión del [PATÓGENO X] y se han aplicado los procedimientos de *desinfección* apropiados (descritos en el Capítulo 4.3.), y

c) las *condiciones elementales de bioseguridad* vigentes anteriormente han sido debidamente revisadas y modificadas y se han reunido ininterrumpidamente desde la erradicación de la infección por el [PATÓGENO X], y

d) se ha aplicado una *vigilancia específica*, de conformidad con lo descrito en el Capítulo 1.4., durante i) por lo menos los [dos] últimos años y no se ha detectado la presencia del [PATÓGENO X] o ii) por lo menos el [último] año y no se ha detectado la presencia del [PATÓGENO X] si las granjas infectadas no estuvieron epidemiológicamente vincualdas a poblaciones silvestres de *especies susceptibles*

Mientras tanto, parte o la totalidad del país, salvo las *zonas infectadas* y las *zonas de protección*, podrá ser declarado una *zona* libre, siempre que reúna las condiciones descritas en el apartado 2 del Artículo X.X.6.

Artículo X.X.6.

[Nota: nuevo artículo para zona libre modificado a partir del Artículo existente X.X.5.]

Zona libre de infección por el [PATÓGENO X]

Si una *zona* se extiende más allá de las fronteras de un país, sólo podrá ser declarada *zona* libre de infección por el [PATÓGENO X] si las *autoridades competentes* confirman que reúne las condiciones exigidas para serlo.

Como se describe en el Artículo 1.4.X., un País Miembro podrá hacer una *autodeclaración de ausencia* de infección por el [PATÓGENO X] para una *zona* dentro de su *territorio* si:

1) ninguna *especie susceptible* de las mencionadas en el Artículo 10.6.2.. está presente y se han reunido ininterrumpidamente las *condiciones elementales de bioseguridad* durante, por lo menos, los [dos] últimos años;

O

2) no ha ocurrido ninguna infección por el [PATÓGENO X] durante, por lo menos, los [diez] últimos años, y:

a) el País Miembro puede demostrar condiciones propicias para la manifestación clínica de la infección por el [PATÓGENO X] , de acuerdo con lo indicado en el capítulo correspondiente del *Manual Acuático*, y

b) se han reunido ininterrumpidamente las *condiciones elementales de bioseguridad* descritas en el Capítulo 1.4. para la *zona* durante, por lo menos, los [10] últimos años;

O

3) se ha aplicado una *vigilancia específica* en la *zona*, de conformidad con lo descrito en el Capítulo 1.4., durante, por lo menos, los [dos] últimos años y no se ha detectado la presencia de [PATÓGENO X] y:

a) se han reunido ininterrumpidamente las *condiciones elementales de bioseguridad* durante, por lo menos, [un] año antes del inicio de la *vigilancia específica*;

O

4) había hecho previamente una *autodeclaración de ausencia* de infección por el [PATÓGENO X] para una *zona* y perdió posteriormente su estatus libre por haberse detectado el [PATÓGENO X], pero se han dado las condiciones siguientes:

   a) en cuanto se detectó el [PATÓGENO X], el área afectada fue declarada *zona infectada* y se estableció una *zona de protección*, y

   b) las poblaciones infectadas dentro de la *zona infectada* se han sacrificado y eliminado con medios que reducen al mínimo la probabilidad de una mayor transmisión del [PATÓGENO X] y se han aplicado los procedimientos de *desinfección* apropiados (descritos en el Capítulo 4.3.), y

   c) las *condiciones elementales de bioseguridad* vigentes anteriormente han sido debidamente revisadas y modificadas y se han reunido ininterrumpidamente desde la erradicación de la infección por el [PATÓGENO X], y

   d) se ha aplicado una *vigilancia específica*, de conformidad con lo descrito en el Capítulo 1.4., durante por lo menos los [dos] últimos años y no se ha detectado la presencia del [PATÓGENO X].

Artículo X.X.7.

[Nota: nuevo artículo dedicado a los compartimentos]

Compartimento libre de infección por el [PATÓGENO X]

Como se describe en el Artículo 1.4.X., un País Miembro podrá hacer una *autodeclaración de ausencia* de infección por el [PATÓGENO X] para un *compartimento* dentro de su *territorio* si:

1) se ha aplicado una *vigilancia específica* en el *compartimento*, de conformidad con lo descrito en el Capítulo 1.4., durante, por lo menos, los [dos] últimos años y no se ha detectado la presencia de [PATÓGENO X] y:

   a) se han reunido ininterrumpidamente las *condiciones elementales de bioseguridad* durante, por lo menos, [un] año antes del inicio de la *vigilancia específica*;

O

2) había hecho previamente una *autodeclaración de ausencia* de infección por el [PATÓGENO X] para un *compartimento* y perdió posteriormente su estatus libre por haberse detectado el [PATÓGENO X] en la *zona*, pero se han dado las condiciones siguientes:

   a) todos los *animales acuáticos* dentro del *compartimento* se han sacrificado y eliminado con medios que reducen al mínimo la probabilidad de una mayor transmisión del [PATÓGENO X] y se han aplicado los procedimientos de *desinfección* apropiados (descritos en el Capítulo 4.3.), y se procedió al *vacío sanitario* en el *compartimento* durante, por lo menos, [X] semanas; y

   b) las *condiciones elementales de bioseguridad* vigentes anteriormente, incluyendo el *plan de bioseguridad*, han sido debidamente revisadas y modificadas y se han reunido ininterrumpidamente desde el momento de la repoblación con animales de fuentes aprobadas libres de patógenos de conformidad con los requisitos del Artículo X.X.9. [*nota: Artículo X.X.7 existente*] y X.X.10 según corresponda; y

   c) se ha aplicado una *vigilancia específica*, de conformidad con lo descrito en el Capítulo 1.4., durante por lo menos el [último] año y no se ha detectado la presencia del [PATÓGENO X].

Artículo X.X.8.

[Nota: modificado del actual Artículo X.X.6]

Conservación del estatus libre

Un país o una *zona* declarados libres de infección por el [PATÓGENO X], de conformidad con lo dispuesto en el apartado 1 de los Artículos X.X.5. or X.X.6 (según proceda), podrán conservar su estatus libre de infección por el [PATÓGENO X] si se mantienen ininterrumpidamente las *condiciones elementales de bioseguridad*.

Un país o una *zona* declarados libres de infección por el [PATÓGENO X], de conformidad con lo dispuesto en el apartado 2 de los Artículos X.X.5. ó X.X.6. (según proceda), podrán interrumpir la *vigilancia específica* y conservar el estatus de país o *zona* libres de infección por el [PATÓGENO X] si se mantienen ininterrumpidamente las condiciones propicias para la manifestación clínica de la infección por el [PATÓGENO X], de acuerdo con lo indicado en el capítulo correspondiente del *Manual Acuático*, y las *condiciones elementales de bioseguridad*.

En un país o una *zona* declarados libres de infección por el [PATÓGENO X], situados dentro del *territorio* de países infectados, se deberá mantener un nivel de *vigilancia específica* que determinará el *Servicio de sanidad de los animales acuáticos* en función de la probabilidad de introducción de la *infección*.

En todos los casos en que no se reúnan condiciones propicias para la manifestación clínica de la infección por el [PATÓGENO X], se requiere que la *vigilancia específica*, descrita en el Capítulo 1.4, mantenga el nivel de confianza en la ausencia de infección por el [PATÓGENO X] que se requirió para la declaración inicial.

EVALUACIÓN DE LA INFECCIÓN POR EL VIRUS ~~1~~ IRIDISCENTE DE LOS DECÁPODOS TIPO 1 PARA INCLUSIÓN EN EL CAPÍTULO 1.3. DEL *CÓDIGO SANITARIO PARA LOS ANIMALES ACUÁTICOS*

Evaluación general

La Comisión de Normas Sanitarias de la OIE para los Animales Acuáticos (la Comisión) evaluó la infección por el virus ~~1~~ iridiscente de los decápodos tipo 1 (DIV1 por sus siglas en inglés) de acuerdo con los criterios para incluir una enfermedad de los animales acuáticos en el Artículo 1.2.2. del *Código Acuático* y acordó que la infección por el virus ~~1~~ iridiscente de los decápodos tipo 1 cumple con los criterios de inclusión en la lista, en particular el criterio 1.: es probable la propagación internacional del agente patógeno; el criterio 2.: al menos un país puede demostrar en el país o en una zona la ausencia de enfermedad en animales acuáticos susceptibles; el criterio 3.: se dispone de una definición de caso precisa y existen métodos de detección y diagnóstico fiables, y el criterio 4b.: se ha demostrado que la enfermedad afecta la sanidad de los animales acuáticos de cultivo a nivel de un país o una zona lo que conlleva consecuencias significativas, por ejemplo, pérdidas de producción, morbilidad o mortalidad (ver Cuadro 1 a continuación).

Cuadro 1. Resumen de la evaluación de la infección por (DIV1)

	Criterios para la inclusión						Conclusión
	1	2	3	4a	4b	4c	
Infección por DIV1	+	+	+	NA	+	-	La enfermedad cumple con los criterios de inclusión en la lista.

NA = no aplica.

Contexto

Se ha identificado un nuevo miembro de la familia *Iridoviridae*, denominado virus ~~1~~ iridiscente de los decápodos tipo 1 (ICTV, 2019), con un genoma de doble cadena de ADN de aproximadamente 166K bp (Li *et al*., 2017; Qiu *et al*., 2017b) como la causa de mortalidades masivas en la producción de camarones, langostinos y cangrejos (Xu *et al*., 2016; Qiu *et al*., 2017a; Qiu *et al*., 2019a). Hasta ahora, la infección por DIV1 se ha detectado exclusivamente en la langosta australiana de agua dulce (*Cherax quadricarinatus*) (Xu *et al*., 2016), el camarón blanco *(Penaeus vannamei)* Qiu *et al.*, 2017), el camarón gigante de agua dulce (*Macrobrachium rosenbergii*) (Qiu *et al*., 2019a), el cangrejo rojo americano (*Procambarus clarkia*) (Qiu *et al*., 2019a), el camarón nipón (*Macrobrachium nipponense*) (Qiu *et al*., 2019a) y el camarón quilla (*Exopalaemon carinicauda*). Se ha demostrado la infección por el virus ~~1~~ iridiscente de los decápodos tipo 1 en dos especies de cangrejos, el cangrejo chino (*Eriocheir sinensis*) y el cangrejo de pantano (*Pachygrapsus crassipes*) por medio de infección experimental por vías no naturales (Pan *et al*., 2017). La Comisión ha reconocido el potencial significativo de este virus en numerosos países dada la importancia mundial del comercio y de la producción de crustáceos. Por el momento, la 1 de los decápodos se considera una "enfermedad emergente" y, como tal, deberá ser notificada de acuerdo con el Artículo 1.1.4. del *Código Acuático*.

Criterios para la inclusión de las enfermedades de los animales acuáticos en la lista de la OIE (Artículo 1.2.2.)

Criterio No. 1. ***Es probable la propagación internacional del agente patógeno (a través de animales acuáticos, sus productos, vectores o fómites).***

Evaluación

El virus se ha detectado por PCR o por el método de la PCR anidada en el camarón blanco *(Penaeus vannamei)*, el camarón gigante de agua dulce (*Macrobrachium rosenbergii*) (Qiu *et al*., 2019a), el cangrejo rojo americano (*Procambarus* ~~*clarkia*~~ *clarkii*) (Qiu *et al*., 2019a), el camarón nipón (*Macrobrachium nipponense*) (Qiu *et al*., 2019a) y el camarón quilla (*Exopalaemon carinicauda*) en granjas de China (Xu *et al*., 2016; Qiu *et al*., 2017a; Qiu *et al*., 2018b; Qiu *et al*., 2019b). Históricamente, *P. vannamei* y otras especies de crustáceos susceptibles se han comercializado internacionalmente como población productora de reserva y postlarva para producción en nuevas regiones geográficas ~~y los productos de *P. vannamei* se comercializan a escala internacional~~. Por lo tanto, existen rutas para la transmisión y la probabilidad de propagación internacional. Los exámenes histopatológicos (observaciones con MET e hibridación *in-situ)* evidencian la presencia del virus en tejidos hematopoyéticos, branquias, hepatopáncreas, pereiopodos y músculos (~~Qui~~ Qiu *et al.*, 2017a). La detección por PRC cuantitativa en el camarón infectado en condiciones experimentales mostró que la concentración viral más alta se encuentra en la hemocianina y en los tejidos hemopoyéticos, mientras que la más baja se localiza en los tejidos musculares (Qiu *et al.*, 2018a; Qiu *et al.*, 2018b).

Conclusión

Se cumple el criterio.

Y

Criterio No. 2. ***Al menos un país puede demostrar en el país o en una zona la ausencia de enfermedad en animales acuáticos susceptibles.***

Evaluación

Actualmente, el virus DIV1 sólo se ha detectado en China (Rep. Pop) pero la distribución geográfica del virus puede ser más extensa ~~de lo que se ha notificado~~ si hay mortalidades que todavía no se han investigado. Sin embargo, ante la amplia distribución de *P. vannamei*, *M. rosenbergii* y de otras especies susceptibles a la infección por DIV1 al igual que ante el importante comercio de estas especies y la posible expresión de enfermedad clínica y mortalidad, si el virus se hubiera propagado ampliamente se hubiese esperado que se notificara la aparición de la enfermedad en otros lugares.

Además, la enfermedad se ha inscrito como una enfermedad de declaración obligatoria en el "Informe trimestral sobre las enfermedades de los animales acuáticos" de la Red de centros de acuicultura de Asia-Pacífico (NACA). Por consiguiente, es probable que al menos un país pueda demostrar la ausencia de enfermedad en su territorio o en una zona en los *animales acuáticos* susceptibles.

Conclusión

Se cumple el criterio.

Y

Criterio No. ***3. Se dispone de una definición de caso precisa y existen métodos de detección y diagnóstico fiables.***

Evaluación

En *P. vannamei* infectados, se constata que el estómago y las vísceras de todos los camarones enfermos estaban vacíos; los camarones parcialmente infectados presentaban una ligera pérdida del color en la superficie y en la sección del hepatopáncreas, además de un caparazón blando. En ~~A~~algunos individuos se observa un ligero enrojecimiento del ~~tenían el~~ cuerpo. Los camarones moribundos pierden la capacidad de nadar y se hunden en el fondo del estanque (Qiu *et al.*, 2017a). En *M. rosenbergii*, los individuos enfermos presentan un triángulo blanco de tamaño significativo dentro del caparazón en la base del rostrum, donde se sitúa el tejido hematopoyético (Qiu *et al*., 2019a).

Hasta la fecha, se han publicado y están disponibles para la detección del virus DIV1 ~~iridiscente del hemocito del~~ un método PCR anidado, un método PCR cuantitativo en tiempo real con una sonda TaqMan (TaqMan qPCR) (Qiu *et al.*, 2018a), un método de hibridación *in situ* (Qiu *et al.*, 2017a) y un ensayo de amplificación de ADN mediado por marcación con DIG (digoxigenina) *in situ* (Chen *et al*., 2019). Se ha demostrado que los cebadores y la sonda TaqMan resultan específicos para el virus DIV1 (ninguna reacción cruzada con otros agentes patógenos del camarón), con un bajo límite de detección (cuatro copias por reacción) y una alta sensibilidad y especificidad (95,3% y 99,2%, respectivamente). Se han validado las pruebas de PCR anidada y de PCR en tiempo real con una sonda TaqMan.

Se puede concluir que ~~a)~~ existen ~~un~~ métodos confiables de detección y diagnóstico, y ~~b)~~ se puede desarrollar una definición de caso precisa en base a signos clínicos observados y ~~c) en el uso~~ de pruebas de diagnóstico disponibles.

Conclusión

Se cumple el criterio.

Y

Criterio No. 4. a. ***Se ha demostrado la transmisión natural de la enfermedad al ser humano y la infección humana se asocia con consecuencias graves.***

Evaluación

Ningún dato disponible para la evaluación.

Conclusión

El criterio no se aplica

O

Criterio No. 4.b. ***Se ha demostrado que la enfermedad afecta la sanidad de los animales acuáticos de cultivo a nivel de un país o una zona lo que conlleva consecuencias significativas, por ejemplo, pérdidas de producción, morbilidad o mortalidad.***

Evaluación

Se ha observado una alta mortalidad (>80%) en las poblaciones afectadas de *P. vannamei* y *M. rosenbergii* en granjas de China (Rep. Pop.) (Qiu *et al*., 2017a; Qiu *et al*., 2019a). Las pruebas de infección experimentales en laboratorio imitando la ruta de infección natural (*per os* ~~*y per anus*~~) en *P. vannamei* han mostrado un 100% de mortalidad acumulada en menos de dos semanas (Qiu *et al.*, 2017a). Pruebas de transmisión por inyección en *P. vannamei*, *C. quadricarinatus* y *P. clarkii* también resultaron en un 100% de mortalidad acumulada (Xu *et al.*, 2016; Qiu *et al.*, 2017a). Desde 2014, algunos episodios de mortalidad masiva de *P. vannamei* y *M. rosenbergii* en las provincias costeras de China (Rep. Pop.) se han asociado con la infección por DIV1 (Qui *et al.*, 2017a). La vigilancia específica en China en 2017 y 2018 ~~relevó que~~ detectó el DIV1 ~~se ha detectado~~ en 11 de 16 provincias (Qiu *et al*., 2018b; Qiu *et al*., 2019b). Las pérdidas son significativas a escala del país.

Conclusión

Se cumple el criterio.

O

Criterio No. 4.c. Se ha demostrado o las pruebas científicas indican que la enfermedad puede afectar la sanidad de los animales acuáticos silvestres lo que conlleva consecuencias significativas, por ejemplo, morbilidad o mortalidad a nivel de la población, productividad reducida o impactos ecológicos.

Evaluación

Se ha demostrado que la infección por DIV1 afecta significativamente la salud de los camarones~~,~~ o cangrejos ~~o langostas~~ de cultivo, con consecuencias graves, sobre todo en cuanto a la morbilidad y la mortalidad. Es posible que la enfermedad también afecte a animales acuáticos silvestres. Sin embargo, hasta la fecha, no existen datos disponibles para demostrar el impacto (por ejemplo, morbilidad o mortalidad) de la enfermedad en las poblaciones de animales acuáticos silvestres.

Conclusión

No se cumple el criterio.

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CHAPTER 2.3.9.

INFECTION WITH SPRING VIRAEMIA OF CARP VIRUS

1. Scope

Infection with spring viraemia of carp virus means infection with the pathogenic agent *Carp sprivivirus* (commonly known as spring viraemia of carp virus [SVCV]), of the Genus *Sprivivirus* and the Family *Rhabdoviridae.*

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

The virus genome is a non-segmented, negative-sense, single strand of RNA. The genome contains 11,019 nucleotides encoding five proteins in the following order: a nucleoprotein (N), a phosphoprotein (P), a matrix protein (M), a glycoprotein (G) and an RNA-dependent, RNA polymerase (L). The genome does not contain a non-virion (NV) gene between the G and L genes as is found in fish rhabdoviruses of the genus *Novirhabdovirus* (Ahne *et al.,* 2002). The type strain of SVCV is available from the American Type Culture Collection (ATCC VR-1390). Two complete genome sequences of the type strain have been submitted to Genbank (Genbank accession U18101 by Bjorklund *et al.* [1996] and Genbank accession AJ318079 by Hoffmann *et al.* [2002]). The complete genome sequence of isolates from China (People's Rep. of) has also been deposited in Genbank (Genbank accession DQ097384 by Teng *et al.* [2007] and Genbank accession EU177782 by Zhang *et al.* [2009]).

Stone *et al.* (2003) used sequence analysis of a 550 nucleotide region of the G-gene to compare 36 isolates from different fish species and geographical locations that were previously identified by serology as SVCV or pike fry rhabdovirus (PFRV)~~ by serology~~. The analysis showed that the isolates could be separated into four distinct genogroups and that all of the SVCV isolates could be assigned to genogroup I, sharing <61% nucleotide identity with viruses in the other three genogroups. Re-analysis of the sequence data generated for viruses assigned to Genogroup I identified four subgroups (Ia–d). Those viruses originating in Asia were assigned to Subgroup Ia, those from Moldova, the Ukraine and Russia to Subgroups Ib and Ic, and those from the UK to Subgroup Id.

2.1.2. Survival and stability in processed or stored samples

There are limited published data on the stability of the pathogen in host tissues. There is also limited information on the stability of the virus in the tissues after death of a diseased animal. Detection of SVCV in the tissues of recently dead animals by either ~~both~~ ~~reverse-transcription polymerase chain reaction (~~RT-PCR~~)~~, or ~~and~~ culture may be possible~~ should not be ruled out~~, and therefore, dead fish ~~as well as moribund should~~ may be taken for analysis if moribund fish are not available.

The virus can be stored for several months when frozen in medium containing 2–5% serum. The virus is most stable at lower temperatures, with little loss of titre ~~for~~ when stored for 1 month at –20°C, or for 6 months at –30 or –74°C (Ahne, 1976; de Kinkelin & Le Berre, 1974). The virus is stable over four freeze (–30°C)–thaw cycles in medium containing 2% serum (de Kinkelin & Le Berre, 1974).

2.1.3. Survival and stability outside the host

The virus ~~has been shown to~~ can remain viable outside the host for 5 weeks in river water at 10°C and for more than 6 weeks in pond mud at 4°C, reducing to 4 days in pond mud at 10°C (Ahne, 1976).

For inactivation methods, see Section 2.4.5.

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with SVCV according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) are: ~~all varieties and subspecies of common carp (*Cyprinus carpio*), bighead carp (*Aristichthys nobilis*), bream (*Abramis brama*), Caspian white fish (*Rutilus kutum*), fathead minnow (*Pimephales promelas*), golden shiner (*Notemigonus crysoleucas*), goldfish (*Carassius auratus*), grass carp (*Ctenopharyngodon Idella*), roach (*Rutilus rutilus*) and sheatfish (also known as European or wels catfish) (*Silurus glanis*).~~

Family	Scientific name	Common name
Cyprinidae	*Abramis brama*	Bream
	Aristichthys nobilis	Bighead carp
	Carassius auratus	Goldfish
	Ctenopharyngodon idella	Grass carp
	Cyprinus carpio	Common carp (all varieties and subspecies)
	Danio rerio	Zebrafish
	Notemigonus crysoleucas	Golden shiner
	Pimephales promelas	Fathead minnow
	Rutilus kutum	Caspian white fish
	Rutilus rutilus	Roach
Siluridae	*Silurus glanis*	~~Sheatfish (also known as European or wels catfish)~~ Wels catfish

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence for susceptibility according to Chapter 1.5. of the *Aquatic Code* are: Crucian carp (*Carassius carassius*), pike (*Esox lucius*), firebelly newt (*Cynops orientalis*), silver carp (*Hypophthalmichthys molitrix*), and yellow perch (*Perca flavescens*) ~~and zebrafish (*Danio rerio*)~~.

~~Evidence is lacking for these species to either confirm that the identity of the pathogenic agent is SVCV, transmission mimics natural pathways of infection, or presence of the pathogenic agent constitutes an infection.~~

~~Family~~	~~Scientific name~~	~~Common name~~
~~Cyprinidae~~	~~*Carassius carassius*~~	~~Crucian carp~~
	~~*Hypophthalmichthys molitrix*~~	~~Silver carp~~
~~Esocidae~~	~~*Esox lucius*~~	~~Northern pike~~
~~Percidae~~	~~*Perca flavescens*~~	~~Yellow perch~~
~~Salamandridae~~	~~*Cynops orientalis*~~	~~Firebelly newt~~

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following organisms, but an active infection has not been demonstrated:

Family	Scientific name	Common name
Catostomidae	*Catostomus commersonii*	White sucker
Cichlidae	~~*Sarotherodon niloticus*~~ *Oreochromis niloticus*	Nile tilapia
Cyprinidae	*Notropis atherinoides*	Emerald shiner
	Cirrhinus mrigala	Mrigal carp
	Labeo rohita	Rohu
	Tinca tinca	Tench
Penaeidae	*Litopenaeus vannamei*	Pacific white shrimp
Salmonidae	*Oncorhynchus tshawytscha*	Chinook salmon
	Oncorhynchus nerka	Sockeye salmon
	Oncorhynchus mykiss	Rainbow trout
	~~*Oncorhynchus mykiss*~~	~~Steelhead trout~~

2.2.3. Non-susceptible species

Species that have been found non-susceptible to infection with SVCV according to Chapter 1.5. of the *Aquatic Code* are: Largemouth bass (*Micropterus salmoides*), Muskellunge (*Esox masquinongy*), and Walleye (*Sander vitreus*).

~~Family~~	~~Scientific name~~	~~Common name~~
~~Centrarchidae~~	~~*Micropterus salmoides*~~	~~Largemouth bass~~
~~Esocidae~~	~~*Esox masquinongy*~~	~~Muskellunge~~
~~Percidae~~	~~*Sander vitreus*~~	~~Walleye~~

2.2.4. Likelihood of infection by species, host life stage, population or sub-populations

Common carp varieties are the principal hosts for SVCV and are considered to be most likely ~~susceptible~~ to be infected ~~infection~~ with SVCV followed~~, in order of susceptibility,~~ by other carp species (including hybrids), other susceptible cyprinid species and finally ~~susceptible~~ non-cyprinid fish species. ~~When sampling during surveillance programmes for SVCV, common carp or strains such as koi or ghost (koi × common) carp are preferentially selected, followed by carp hybrids (e.g. common carp × crucian carp), then other carp species such as crucian carp, goldfish, grass carp, bighead carp and silver carp. Should these species not be available then other known susceptible species should be sampled. Cyprinid species may be are increasingly mixed together in polyculture systems and the risk of transmission of SVCV between species during disease outbreaks is high (Billard & Berni, 2004).~~

Generally, young fish up to one-year old are most ~~susceptible to~~ likely to demonstrate clinical signs of disease, but all age groups can be affected. Moreover, there is a high variability in the degree of susceptibility to infection with SVCV among individuals of the same fish species. Apart from the physiological state of the fish, the role of which is poorly understood, age or the age-related status of innate immunity appears to be extremely important to the manifestation of clinical disease: ~~the~~ younger ~~the~~ fish~~, the higher the susceptibility~~ are more likely to show signs of overt disease~~, although even adult broodfish can be susceptible to infection~~.

Fish that have separated from the shoal and found at the water inlet or sides of a pond are more likely to be infected.

2.2.5. Distribution of the pathogen in the host

~~The transmission of SVCV is horizontal (Fijan, 1988).~~ SVCV appears to enter via the gills and then spread~~s~~ to the kidney, liver, heart, spleen and alimentary tract. During disease outbreaks high titres of virus occur in the liver and kidney of infected fish, but much lower titres occur in the spleen, gills and brain (Dixon, 2008). The virus has been detected in ovarian fluid (Bekesi & Csontos, 1985)~~, but vertical transmission has yet to be demonstrated~~.

2.2.6. Aquatic animal reservoirs of infection

Liu *et al.* (2004) isolated SVCV in China (People's Rep. of) from common and koi carp exhibiting no external or internal signs of disease, and similarly, the virus was isolated from apparently healthy wild carp in Canada (Garver *et al*., 2007). Thus fish surviving infection with SVCV may act as reservoirs of infection.

2.2.7. Vectors

The parasitic invertebrates *Argulus foliaceus* (Crustacea, Branchiura) and *Piscicola geometra* (Annelida, Hirudinea) have been demonstrated to transfer SVCV from diseased to healthy fish under experimental conditions and the virus has been isolated from *A. foliaceus* removed from infected carp (Ahne *et al.*, 2002; Dixon, 2008). It has been demonstrated experimentally that virus can be isolated from fish tissues regurgitated by herons (*Ardea cinerea*) 120 minutes after being fed with SVCV-infected carp, suggesting a potential route for SVCV transmission, but is not known whether such transmission has occurred in nature (Peters & Neukirch, 1986).

2.3. Disease pattern

2.3.1. Mortality, morbidity and prevalence

A noticeable increase in mortality will occur in the population during an outbreak of infection with SVCV ~~there will be a noticeable increase in mortality in the population~~. ~~Co-infections with koi herpesvirus or carp oedema virus can increase levels of mortality.~~ Disease patterns are influenced by water temperature, age and condition of the fish, population density and stress factors. The immune status of the fish is also an important factor with both nonspecific (e.g. interferon) and specific immunity (serum antibodies, cellular immunity) having important roles. Poor physiological condition of over-wintered fish may be a contributory factor to the onset of clinical disease in infected animals ~~susceptibility~~. In European aquaculture, losses can be up to 70% in young carp (Ahne *et al.*, 2002), but are usually from 1 to 40%.

In one survey from Serbia, the virus was isolated by culture in samples collected from 12 of the 38 hatcheries screened over the 10-year period (1992–2002) (Svetlana *et al.,* 2004). The virus occurred sporadically in different ponds on one site, and sporadically from year to year at different sites (Svetlana *et al.,* 2004). In another study, 18 of 30 tissue pools (five fish/pool) of wild, clinically health, common carp sampled in Canada in 2006 were positive for SVCV by culture (Garver *et al.,* 2007). ~~The isolation of SVCV in the latter case was from asymptomatic common carp which correlates with~~ This observation suggests that SVCV infection may ~~can often~~ be clinically inapparent (Fijan, 1999).

2.3.2. Clinical signs, including behavioural changes

Fish can become lethargic, separate from the shoal and gather at the water inlet or sides of a pond and some may experience loss of equilibrium. Clinical signs of infection with SVCV are nonspecific and not all fish will exhibit all of the signs. Two of the most obvious and consistent features are abdominal distension and haemorrhages, which ~~. The latter~~ may be pale and occur on the skin, fin bases, eyes and gills ~~, which may be pale~~. The skin may darken and exophthalmia is often observed. The vent may be swollen, inflamed and trail mucoid casts. ~~During an outbreak of infection with SVCV there will be a noticeable increase in mortality in the population. Diseased fish usually appear darker in colour.~~ There may be no clinical signs in cases with a sudden onset of mortality.

2.3.3 Gross pathology

There are no pathognomonic gross lesions. Lesions may be absent in cases of sudden mortality. Gross pathologies are mainly documented for common carp and may include excess ascitic fluid in the abdominal cavity, usually containing blood, degeneration of the gill lamellae and inflammation of the intestine, which contains mucous instead of food. Oedema and haemorrhage of the visceral organs is commonly observed (the spleen is often enlarged), and organs adhere to each other and to the peritoneum. Focal haemorrhages may be seen in the muscle and fat tissue, as well as in the swim bladder (see Dixon, 2008). However, petechial haemorrhages are infrequent ~~uncommon~~ in cases caused by Asian strains of SVCV (Dikkeboom *et al*., 2004).

2.3.4. Modes of transmission and life cycle

The transmission of SVCV is horizontal (Fijan, 1988). Horizontal transmission may be direct, or via water, fomites or vectors (Section 2.2.7.) (Fijan, 1988). The virus appears to enter the host via the gills. A viraemia follows and the virus rapidly spreads to the liver, kidney, spleen and alimentary tract. The virus can be detected in faeces and is also shed into the water via faeces and urine (Ahne, 1982).

Vertical ~~or~~ and 'egg-associated' transmission cannot be ruled out following one report of isolation of SVCV from carp ovarian fluid, although there have been no further reports (Bekesi & Csontos, 1985).

~~Horizontal transmission may be direct or vectorial, water being the major abiotic vector (Fijan, 1988). Animate vectors (Section 2.2.6.) and fomites may also be involved in transmission of SVCV (Fijan, 1988).~~ Once SVCV is established in populations, it may be very difficult to eradicate without destroying all susceptible species and vectors ~~types of life at the site~~.

2.3.5. Environmental and management factors

Disease outbreaks in carp generally occur between 11 and 17°C. They rarely occur below 10°C, and mortalities, particularly in older fish, decline as the temperature exceeds 22°C (Fijan, 1988). However, the virus was isolated from apparently healthy fish from a lake in Canada that had been sampled over a 13-day period during which the water temperature varied between 24.2°C and 27.3°C (Garver *et al,* 2007). These fish may have been more susceptible to infection as they were penned and detection was during spawning. Secondary and concomitant bacterial ~~and/~~or parasitic infections can affect the mortality rate and ~~display~~ the appearance of clinical signs. In carp, the disease is often observed during ~~in~~ spring~~time~~ (hence the common name for the disease), particularly in countries having cold winters. It is believed that the poor condition of the over-wintered fish may be a contributory factor in the~~disease~~ occurrence of clinical disease. Clinical ~~The~~ disease can occur in fish in quarantine following the stress of transportation, even though there has been no evidence of infection prior to transportation.

2.3.6. Geographical distribution

For a long time, the geographical range of SVCV was limited to countries of the European continent that experience low water temperatures during winter. ~~Consequently~~ The disease has been recorded from most European countries, ~~and from certain of the western Independent States of the former Soviet Union (Belarus, Georgia, Lithuania, Moldova, Russia and the Ukraine) (see Dixon 2008 for references to these and the following locations).~~ However, in 1998, the disease was recorded in South America ~~(in goldfish in a lake in Brazil)~~ and in 2002 in ~~the USA,~~ North America. ~~and in 2006 in Canada~~ The virus was first detected in Asia ~~Detection of the virus in carp in China (People's Rep. of) was confirmed~~ in 2004.

For recent information on distribution at the country level consult the WAHIS interface (https://www.oie.int/wahis_2/public/wahid.php/Wahidhome/Home/index/newlang/en).

2.4. Biosecurity and disease control strategies

2.4.1. Vaccination

A safe and effective vaccine is not currently available; however, ~~a number~~ the efficacy of an experimental DNA vaccine has been investigated ~~inactivated preparations, live attenuated vaccines and DNA vaccines have given encouraging results (Dixon, 2008,~~ (Emmenegger & Kurath, 2008). ~~The use of live attenuated vaccines or the DNA vaccines might affect diagnostic performance.~~

2.4.2. Chemotherapy including blocking agents

Methisoprinol inhibits the replication of SVCV *in vitro*, but has not been tested under carp culture conditions (Siwicki *et al.,* 2002).

2.4.3. Immunostimulation

Injection into carp of single-stranded and double-stranded RNA (which is an interferon inducer) protected carp for longer than 3 weeks, but the treatment is not effective by bath administration (Alikin *et al.,* 1996).

2.4.4. Breeding resistant strains

The "Krasnodar" strain of common carp has been bred for increased resistance to SVCV (Kirpichnikov *et al*., 1993).

2.4.5. Inactivation methods

The virus is inactivated at 56°C for 30 minutes, at pH 12 for 10 minutes and pH 3 for 2 hours (Ahne, 1986). Oxidising agents, sodium dodecyl sulphate, non-ionic detergents and lipid solvents are all effective for inactivation of SVCV. The following disinfectants are ~~also effective for~~ inactivate the virus~~ion~~: 3% formalin for 5 minutes, 2% sodium hydroxide for 10 minutes, 540 mg litre^{-1} chlorine for 20 minutes, 200–250 ppm (parts per million) iodine compounds for 30 minutes, 100 ppm benzalkonium chloride for 20 minutes, 350 ppm alkyltoluene for 20 minutes, 100 ppm chlorhexidine gluconate for 20 minutes and 200 ppm cresol for 20 minutes (Ahne, 1982; Ahne & Held, 1980; Kiryu *et al.,* 2007).

2.4.6. Disinfection of eggs and larvae

Eggs can be disinfected by iodophor treatment (Ahne & Held, 1980).

2.4.7. General husbandry

Methods to control ~~of~~ infection with SVCV rely ~~relies~~ on avoiding exposure to the virus coupled with good hygiene practices. This is feasible on small farms supplied by spring or borehole water and a secure system to prevent fish entering the farm via the discharge water. ~~Hygiene measures should include disinfection of eggs by iodophor treatment (Ahne & Held, 1980), until it has been confirmed unequivocally that vertical transmission does not occur, regular disinfection of ponds, chemical disinfection of farm equipment, careful handling of fish to avoid stress and safe disposal of dead fish.~~ Reducing fish stocking density during winter and early spring will reduce the spread of the virus. In rearing facilities with a controlled environment, elevation of water temperature above 19–20°C may stop or prevent outbreaks of infection with SVCV.

3. Specimen selection, sample collection, transportation and handling

This Section draws on information in Sections 2.2., 2.3. and 2.4. to identify populations, individuals and samples which are most likely to be infected.

3.1. Selection of populations and individual specimens

~~Sampling samples, target comprise of susceptible species on the site with each group being represented in the sample. A group is defined as a. The population to be sampled may be stratified into groups of the same fish species that shares a common water supply and originate from the same broodfish or spawning population. Generally young Moribund fish up to 1 year old are most susceptible to clinical disease, but all age groups can be affected. Any moribund fish present in the fish population to be sampled should be sampled selected first for sample collection and the remainder of the samples should comprise randomly selected live fish from all groups of susceptible species rearing units that represent the lot being examined.~~

Clinical inspections should be carried out during a period when the water temperature is between 11°C and 17°C. All production units (ponds, tanks, net-cages, etc.) should be inspected for the presence of dead, weak or abnormally behaving fish. Particular attention should be paid to the water outlet area where weak fish tend to accumulate due to the water current.

For the purposes of disease surveillance, fish to be sampled are selected as follows:

i) Common carp or strains such as koi or ghost (koi × common) carp are preferentially selected, followed by carp hybrids (e.g. common carp × crucian carp), then other carp species such as crucian carp, goldfish, grass carp, bighead carp and silver carp. Susceptible species should be sampled proportionally, or following risk-based criteria for targeted selection of lots or populations with a history of abnormal mortality or potential exposure events (e.g. via untreated surface water, wild harvest or replacement with stocks of unknown disease status).

ii) If more than one water source is used for fish production, fish from all water sources should be included in the sample.

iii) If weak, abnormally behaving or freshly dead (not decomposed) fish are present, such fish should be selected. If such fish are not present, the fish selected should include normal appearing, healthy fish collected in such a way that all parts of the farm as well as all year classes are proportionally represented in the sample.

For disease outbreak investigations, moribund fish or fish exhibiting clinical signs of infection with SVCV should be collected. Ideally fish should be collected while alive, however, recently dead fish can also be selected for diagnostic testing ~~purposes~~. It should be noted however, that there will be a significant risk of contamination with environmental bacteria if the animals have been dead for some time. There may be no clinical signs or gross pathognomonic lesions ~~and no clinical signs~~ in cases of sudden mortality ~~(see Section 4.1.1.)~~.

3.2. Selection of organs or tissues

Kidney, spleen, gill and encephalon should be selected from ~~subclinically infected fish (~~apparently healthy fish~~)~~.

For clinically affected fish: whole fry ~~alevin~~ (body length ≤ 4 cm), entire viscera including kidney and ~~encephalon~~ brain (> 4 cm body length ≤ 6 cm) or, for larger ~~sized~~ fish, liver, kidney, spleen and encephalon should be selected.

3.3. Samples or tissues not suitable for pathogen detection

~~Virus isolation may also not be possible from~~ Decomposed clinical samples ~~A number of studies in which attempts were made to isolate virus from reproductive fluids were unsuccessful, although~~ and seminal fluid samples are not suitable. While the virus has been isolated at low frequency from ovarian~~, but not seminal,~~ fluids, the suitability of these tissues for detection of SVC ~~samples~~ has not been substantiated (Bekesi & Csontos, 1985).

3.4. Non-lethal sampling

Serological assays for antibodies can be undertaken on blood samples~~; the~~ and can indicate possible exposure to SVCV, however, serology is not a suitable test for making a suspect diagnosis. ~~can only be used for a presumptive diagnosis given cross reactivity of anti-SVCV antibodies with viruses of the species pike fry sprivivirus allows for a presumptive indication of infection with SVCV.~~

3.5. Preservation of samples for submission

For guidance on sample preservation methods for the intended test methods, see Chapter ~~2.2.0 or~~ 2.3.0 ~~or 2.4.0~~.

3.5.1. Samples for pathogen isolation

Samples for virus isolation (Section 3.2.) should be transported to the laboratory at 4°C using refrigerated containers or on ice, preferably in virus transport medium and tested within 24 hours or, in exceptional circumstances, 48 hours. The shipment of organ samples is preferred, but live or whole dead fish can be submitted to the testing laboratory if necessary. If this is not possible, samples can be frozen, but there may be loss of virus viability on thawing the samples. Repeated freeze–thawing of the sample must be avoided.

3.5.2. Preservation of ~~Fixed~~ samples for molecular detection

~~Tissue samples for PCR testing should be preserved in 70–90% (v/v) analytical/reagent-grade (absolute) ethanol. The recommended ratio of ethanol to tissue is 10:1 based on studies in terrestrial animal and human health. The use of lower grade (laboratory or industrial grade) ethanol is not recommended. [Alternatives to ethanol can be mentioned if they can be referenced.]~~

The material collected for virus culture is generally used for the molecular diagnostic assays, but additional tissue samples for RT-PCR can be preserved in commercially available RNA preservation solutions according to the manufacturers' recommendations, or, alternatively, samples can be preserved in 80–90% (v/v) analytical grade (absolute) ethanol at the recommended ratio of ethanol to tissue of 10:1.

3.5.3. Fixed samples for histopathology, immunohistochemistry or *in-situ* hybridisation

Histology samples from each individual fish must be ~~taken~~ placed into 10% neutral buffered formalin ~~(NBF)~~ immediately after collection to prevent sample deterioration. The recommended ratio of fixative to tissue is 10:1 and each sample should be cut cleanly and be no thicker than approximately 4 mm to allow the fixative to penetrate the material ~~and should be cut cleanly~~.

3.5.4. Fixed samples for electron microscopy

~~EM sampling is~~ Samples for electron microscopy are not routinely required ~~as standard, and the material is~~ and are collected only when ~~where~~ it is considered beneficial to facilitate ~~potential~~ further diagnostic investigation ~~work~~. From each fish sampled, a 2 mm cubed (approximately) sample ~~section~~ from each of the appropriate organs described in section 3.2 should be fixed in glutaraldehyde; the recommended ratio of fixative to tissue is 10:1.

3.5.5. Samples for other tests

Tubes for the separation of serum are available commercially. After collection, the blood is allowed to clot by leaving it undisturbed at room temperature. This usually takes 15–30 minutes. Serum is clarified by centrifuging at 1000–2000 ***g*** for 10 minutes ~~in a refrigerated centrifuge~~ at 4–8°C.

~~It is important to immediately transfer the liquid component (serum) into a clean polypropylene tube using a Pasteur pipette and maintain the samples at 2–8°C while handling. If the serum is not analysed immediately, it should be apportioned into 0.5 ml aliquots, stored, and transported at –20°C or lower. It is important to avoid freeze–thaw cycles because this is detrimental to many serum components. Samples that are haemolysed, icteric or lipaemic can invalidate certain tests.~~

3.6. Pooling of samples

~~Traditionally pools of five animals have been used and more recently this has been increased to pools of ten animals for virus culture. However, no published data on the effect of pooling on test characteristics has been published.~~

Pooling of samples from more than one individual animal for a given purpose should only be recommended where supporting data on diagnostic sensitivity and diagnostic specificity are available. However, smaller life stages (e.g. fry) can be pooled to provide a minimum amount of material for testing.

4. Diagnostic methods

The methods currently available for identifying infection that can be used in i) surveillance of apparently healthy populations), ii) presumptive and iii) confirmatory diagnostic purposes are listed in Table 4.1. by life stage. The designations used in the Table indicate:

Key:

+++ =	Recommended method(s) validated for the purpose shown and usually to stage 3 of the OIE Validation Pathway;
++ =	Suitable method(s) but may need further validation;
+ =	May be used in some situations, but cost, reliability, lack of validation or other factors severely limits its application;
Shaded boxes =	Not appropriate for this purpose.

The selection of a test for a given purpose depends on the analytical and diagnostic sensitivities and specificities, repeatability and reproducibility. OIE Reference Laboratories welcome feedback on diagnostic performance for assays, in particular PCR methods, for factors affecting assay analytical sensitivity or analytical specificity, such as tissue components inhibiting amplification, presence of nonspecific or uncertain bands, etc., and any assays that are in the +++ category.

Table 4.1. *OIE recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals*

Method	A. Surveillance of apparently healthy animals				B. Presumptive diagnosis of clinically affected animals				C. 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
Wet mounts												
Histopathology[3]												
Cytopathology[3]												
Cell or artificial media culture		++	++	~~1~~ 3		++	++	~~1~~ 3		++	++	~~1~~ 3
Real-time PCR												
Conventional PCR		++	++	~~1~~ 2		++	++	~~1~~ 2		++	++	~~1~~ 2
Amplicon sequencing[4]										+++	+++	~~1~~ 3
In-situ hybridisation												
Immunohistochemistry						++	++	1				
Bioassay												
LAMP												
Ab-ELISA												
Ag-ELISA						++	++	1				
IFAT ~~Other antigen detection methods~~						++	++	1				

LV = level of validation, refers to the stage of validation in the OIE Pathway (chapter 1.1.2); PCR = polymerase chain reaction; LAMP = loop-mediated isothermal amplification; Ab- or Ag-ELISA = antibody or antigen enzyme-linked immunosorbent assay.

[1]For confirmatory diagnoses, methods need to be carried out in combination (see Section 6).

[2]Early and juvenile life stages have been defined in Section 2.2.4.

[3]Histopathology and cytopathology can be validated if the results from different operators have been statistically compared.

[4]Sequencing of the PCR product.

Shading indicates the test is inappropriate or should not be used for this purpose.

4.1. Wet mounts

Not applicable.

4.2. Histo-pathology and cytopathology

Histopathological changes can be observed in all major organs. In the liver, blood vessels show oedematous perivasculitis progressing to necrosis. Liver parenchyma shows hyperaemia with multiple focal necrosis and degeneration. The heart shows pericarditis and infiltration of the myocardium progressing to focal degeneration and necrosis. The spleen shows hyperaemia with hyperplasia of the reticuloendothelium and enlarged melanomacrophage centres, and the pancreas is inflamed with multifocal necrosis. In the kidney, damage is seen to excretory and haematopoietic tissue. Renal tubules are clogged with casts and the cells undergo hyaline degeneration and vacuolation. The intestine shows perivascular inflammation, desquamation of the epithelium and atrophy of the villi. The peritoneum is inflamed, and lymph vessels are filled with detritus and macrophages. In the swim bladder, the epithelial lamina changes from a monolayer to a discontinuous multi-layer and vessels in the submucosa are dilated with nearby lymphocyte infiltration.

As the histopathological presentation ~~picture~~ is not specific for the disease, and not all fish will exhibit each feature (Misk *et al.,* 2016), microscopic methods by themselves are not recommended for diagnosis of SVC ~~as the histopathological picture is not specific for the disease~~. They may, however, provide supporting evidence, particularly, when immunohistochemistry ~~immunohistological (IHC)~~ or nucleic acid ~~DNA~~ based *in-situ* hybridisation methods are used (see the relevant Sections below).

~~Fixed sections can also be used for histoimmunochemistry (but see caveats in Section 4.6.).~~

4.3. Cell or artificial media culture for isolation

4.3.1. Cell lines

~~If culturing viruses~~ The recommended cell lines for SVCV detection are EPC, FHM or GCO. Cell lines should be monitored to ensure that susceptibility to targeted pathogens has not changed.

4.3.2. Sample preparation and inoculation

~~*Cell culture*~~

~~Cell line to be used: EPC, FHM or GCO.~~

Virus extraction: Use the procedure described in Section ~~A.~~2.2.2 of Chapter 2.3.0.

Inoculation of cell monolayers: make two serial tenfold dilutions of the 1/10 organ homogenate supernatants in cell culture medium (i.e. the homogenate supernatants will be 1/100 and 1/1000 dilutions of the original organ material) and transfer an appropriate volume of each of these two dilutions on to 24-hour-old cell monolayers drained of their culture medium. Alternatively, make a single tenfold dilution of the 1/10 organ homogenate (i.e. a 1/100 dilution of the original organ material) and add an appropriate volume of both the 1/10 and 1/100 dilutions directly to undrained 24 hour-old cell monolayers, to effect 1/100 and 1/1000 final dilutions of the organ homogenate. Should toxicity of the sample be a problem, make two serial tenfold dilutions of the 1/10 organ homogenate supernatants in cell culture medium as described above and inoculate at least 2 cm^2 of drained cell monolayer with 100 µl of each dilution. Allow to adsorb for 0.5–1 hour at 10–15°C, withdraw the inoculum and add cell culture medium buffered at pH 7.6 and supplemented with 2% fetal calf serum (FCS) (1 ml $well^{-1}$ for 24-well cell culture plates). Incubate at 20°C.

Monitoring incubation: Follow the course of infection in positive controls and other inoculated cell cultures by microscopic examination at ×40–100 magnification for 7 days. The use of a phase-contrast microscope is recommended.

Maintain the pH of the cell culture medium at between 7.3. and 7.6. during incubation. This can be achieved by the addition to the inoculated medium of sterile bicarbonate buffer (for tightly closed cell culture flasks) or HEPES-buffered medium (HEPES = N-2-hydroxyethyl-piperazine-N-2-ethanesulfonic acid) or 2 M Tris (Tris [hydroxymethy]) aminomethane)/HCl buffer solution (for cell culture plates).

If a cytopathic effect (CPE) appears in those cell cultures inoculated with the dilutions of the tested homogenate supernatants, identification procedures must be undertaken immediately ~~(see Section 4.6.2.)~~.

Subcultivation procedures: Using a pipette, try to dislodge cells from the cell culture vessels and collect aliquots of cell culture medium plus cells from all inoculated monolayers, keeping different groups separate. The aliquots of the 1/100 and 1/000 dilutions are pooled and inoculated on to fresh 24 hour-old cell cultures to effect 1/10 and 1/100 final dilutions of the pooled aliquots. Incubate and monitor as described above. ~~If no CPE occurs, the test may be declared negative.~~

If no CPE occurs the test may be declared negative. However, if undertaking surveillance to demonstrate freedom from SVCV it would be advisable to screen the cells at the end of the 14 days using an SVCV-specific RT-PCR ~~or real-time RT-PCR~~ (Section 4.4.). Following a positive result culture should be re-attempted.

Following isolation, the virus must be identified, and this can be achieved by antigen detection methods, virus neutralisation or nucleic acid identification methods. The former two methods are generally regarded as presumptive unless fully validated monoclonal or polyclonal antibodies are used, as cross reactions with other viruses occur. Commercially available kits using polyclonal antibodies may also lack specificity, and those using monoclonal antibodies may not detect all subgenogroups of SVCV (Dixon & Longshaw, 2005). Nucleic acid detection methods must always be followed up by sequencing or use of a method such as reverse hybridisation (Sheppard *et al*., 2007) to confirm the identity of the virus.

4.4. Nucleic acid amplification

4.4.1. Real-time PCR

The following controls should be run with each assay: negative extraction control; positive control; no template control; internal PCR control if available and validated.

Real-time RT-PCR assays are available to detect and confirm ~~infection with~~ SVCV (Yue *et al*., 2008; Zhang *et al*., 2009), however, they are not currently recommended as they have not been sufficiently validated.

4.4.2. Conventional PCR

~~The following controls should be run with each assay: negative extraction control; positive control; no template control; internal PCR control.~~ Positive and negative controls should be run with each stage of the assays: extraction, RT-PCR and second round PCR. Due to the sensitive nature of PCR-based assays it is highly recommended that master mix, template addition and PCR amplification occur in designated hoods or spatially separated areas.

Nested reverse-transcription polymerase chain reaction (*RT-PCR*) (*confirmation of virus identity from cell culture isolation or directly from fish tissue extracts*)

The genome of SVCV consists of a single strand of RNA of approximately 11 kb, with negative polarity. Amplification of a 714 bp fragment of SVCV cDNA is performed using primers derived from sequences of the region coding for the glycoprotein gene: 5'-TCT-TGG-AGC-CAA-ATA-GCT-CAR*-R*TC-3' (SVCV F1) and 5'-AGA-TGG-TAT-GGA-CCC-CAA-TAC-ATH*-ACN*-CAY*-3' SVCV R2), using a modification of the method of Stone *et al.* (2003).

i) Total RNA is extracted from 100 µl of supernatant from cell cultures exhibiting CPE or 50 µl of fish tissue extract and dissolved in 40 µl molecular biology grade DNase- and RNase-free water.

   A number of total RNA extraction kits are available commercially that will produce high quality RNA suitable for RT-PCR. ~~Examples are Trizol ReagentT (RL, Life Technologies, Paisley, UK), SV Total RNA isolation system (Promega) and Nucleospin® RNA (AB gene), EZ virus mini kit, Ez RNA tissue mini kit (Qiagen).~~

ii) For cDNA synthesis, a reverse transcription reaction is performed at 37°C for 1 hour in a 20 µl volume consisting of 1 × M-MLV RT reaction buffer (50 mM Tris, pH 8.3, 75 mM KCl, 10 mM DTT, 3 mM $MgCl_2$) containing 1 mM dNTP, 100 pmol SVCV R2 primer, 20 units M-MLV reverse transcriptase (Promega, Southampton, UK) or an equivalent reverse transcriptase system and 1/10 of the total RNA extracted above.

iii) PCR is performed in a 50 µl reaction volume 1 × PCR buffer (50 mM KCl, 10 mM Tris/HCl, pH 9.0, and 0.1% Triton X-100) containing 2.5 mM $MgCl_2$, 200 µM dNTPs, 50 pmol each of the SVCV R2 and SVCV F1 primers, 1.25 units of Taq DNA polymerase, and 2.5 µl reverse transcription reaction mix. The reaction mix is subjected to 35 temperature cycles of: 1 minute at 95°C, 1 minute at 55°C and 1 minute at 72°C followed by a final extension step of 10 minutes at 72°C. Amplified DNA (714 bp) is analysed by agarose gel electrophoresis.

iv) If the CPE in culture is not extensive it is possible that a visible product will not be generated using a single round of amplification. To avoid such problems, use the semi-nested assay using primers: 5'-TCT-TGG-AGC-CAA-ATA-GCT-CAR*-R*TC-3' (SVCV F1) and 5'-CTG-GGG-TTT-CCN*-CCT-CAA-AGY*-TGY*-3' (SVC R4) according to Stone *et al.* (2003).

v) The second round of PCR is performed in a 50 µl reaction volume 1 × PCR buffer (50 mM KCl, 10 mM Tris/HCl, pH 9.0, and 0.1% Triton X-100) containing 2.5 mM $MgCl_2$, 200 µM dNTPs, 50 pmol each of the SVCV R4 and SVCV F1 primers, 1.25 units Taq DNA polymerase, and 2.5 µl of the first round product. The reaction mix is subjected to 35 temperature cycles of: 1 minute at 95°C, 1 minute at 55°C and 1 minute at 72°C followed by a final extension step of 10 minutes at 72°C. Amplified DNA (606 bp) is analysed by agarose gel electrophoresis.

vi) All amplified products are confirmed as SVCV in origin by sequencing, and the SVCV subtype (Ia-Id) is identified using a BLAST search (http://www.ebi.ac.uk/blastall/index.html) or by phylogenetic analysis using the SVCV sequences available in public sequence databases. Phylogenetic analysis is undertaken using a 426 bp region corresponding to nucleotides 429–855 of the glycoprotein gene.

vii) In cases where the CPE is extensive and the virus replicates to a high titre, or where a semi-nested RT-PCR assay was used, sufficient PCR amplicon will be available for direct sequencing. Where the amplified product is weak it is recommended that the product be inserted into an appropriate sequencing vector (e.g. pGEM-T, pCR® 4-TOPO®) prior to undertaking the sequencing. At least two independent amplification and sequencing events should be undertaken to eliminate potential sequence errors introduced by the Taq polymerase.

~~The following controls should be run with each assay: negative extraction control; positive control; no template control; internal PCR control.~~

~~**NOTE:** The appropriate IUB codes have been used where appropriate and are indicated by an asterisk (*).~~

Reverse-transcription polymerase chain reaction (*RT-PCR*) (*confirmation of virus identity*)

Additional conventional RT-PCR assays are available to detect and confirm SVCV infections (Koutna *et al*., 2003; Shimahara *et al*., 2016). A generic primer set based on the polymerase gene also identifies viruses from both the *Sprivivirus* and *Perhabdovirus* genera and can be used to screen a virus culture (Ruane *et al*., 2014). With the exception of the conventional PCR assay developed by Shimahara *et al*. (2016) the other assays were not sufficiently ~~fully~~ validated against representatives from each of the recognised SVCV genogroups and they may fail to detect the full range of SVCV genotypes.

A summary of the Shimahara *et al.* (2016) RT-PCR method follows. Amplification of a 369 bp fragment of SVCV glycoprotein gene is performed using primers as follows: SVCV-G1: 5'-TGA-AGA-YTG-TGT-CAA-TCA-AGTC-3' and SVCV-G2: 5'-GCG-ART-GCA-GAG-AAA-AAG-TG-3'. Preparation of RNA template is the same as nested RT-PCR above. Reverse transcription of SVCV RNA and amplification of cDNA are carried out using SuperScript III one-step RT-PCR with PlatinumR Taq (Invitrogen) according to the manufacturer's instructions. The RT-PCR reaction mixture contained 10 pmol of each primer, 12.5 µl of 2× reaction mix, 1 µl of SuperScript III RT/Platinum *Taq* Mix and 2.5 µl template. After reverse transcription at 50°C for 30 minutes and 94°C for 2 minutes, 40 amplification cycles of 94°C for 15 seconds, 56°C for 30 seconds and 68°C for 1 minute followed by a final extension step at 68°C for 7 minutes is performed. All amplified products are confirmed as SVCV in origin by sequencing.

4.4.3. Other nucleic acid amplification methods

Loop-mediated isothermal amplification assays are available to detect and confirm SVCV infections (Shivappa *et al.,* 2008), however, they are currently not recommended as they are not sufficiently validated.

Infection with SVCV has also been ~~confirmed~~ detected using RT-PCR and hybridisation with non-radioactive probes to determine the genotype (~~Oreshkova *et al.*, 1999;~~ Sheppard *et al*., 2007), however, it is currently not recommended as it is not sufficiently validated.

4.5. Amplicon sequencing

~~See above (Section 4.4.2). All~~ Nucleotide sequencing of all RT-PCR amplicons ~~should be sequenced to confirm that they are SVCV in origin~~ (Section 4.4.2) is recommended as one of the final steps for confirmatory diagnosis. SVCV-specific ~~products~~ sequences will share a higher degree of nucleotide ~~identity~~ similarity to one of the published reference sequences for SVCV (Genbank accession U18101, AJ318079, DQ097384 and EU177782) compared to the published reference sequences for the *Pike spriviviruses* (GenBank FJ872827, KC113518 and KC113517).

4.6. *In-situ* hybridisation ~~(and histoimmunochemistry)~~

Although ~~*In*~~*in-situ* hybridisation can be used to locate SVCV ~~the virus~~ in different tissues ~~on~~ in known positive animals, ~~but~~ this assay is currently not recommended as it has not been ~~well~~ validated as a diagnostic tool for the detection of SVCV ~~as a diagnostic tool~~.

4.7. Immunohistochemistry

SVCV can be detected by immunohistochemistry, however, care must be taken with interpreting the results of ~~serological~~ these tests for SVCV, and positive results ~~from antibody-based assays~~ should be confirmed by RT-PCR and sequencing ~~(see Section 4.8.)~~.

i) Bleed the fish thoroughly.

ii) Make kidney imprints on cleaned glass slides or at the bottom of the wells of a plastic cell culture plate.

iii) Store and transport the kidney pieces as indicated in Section 2.2.1. of Chapter 2.3.0. together with the other organs required for virus isolation.

iv) Allow the imprint to air-dry for 20 minutes.

v) Fix with cold acetone (stored at –20°C) for glass slides or 80% acetone in water or 30% acetone in ethanol, also at –20°C, for plastic wells. Let the fixative act for 15 minutes. Allow the imprints to air-dry for at least 30 minutes and process immediately or freeze at –20°C.

vi) Rehydrate the imprints if they have been stored frozen by four rinsing steps with PBS containing 0.05% Tween 20 (PBST), and remove this buffer completely after the last rinse. Block with 5% skim milk or 1% bovine serum albumin, in PBST for 30 minutes at 37°C.

vii) Rinse four times with PBST, 5 minutes for each rinse. The slides or plastic culture plates can be gently agitated during the rinses.

viii) Prepare a solution of purified antibody or serum to SVCV in PBST, at the appropriate dilution (which has been established previously or as given by the reagent supplier).

ix) Incubate the imprints with the antibody solution for 1 hour at 37°C in a humid chamber and do not allow evaporation to occur.

x) Rinse four times with PBST.

xi) Incubate the imprints with a solution of fluorescein isothiocyanate (FITC)-conjugated antibody to the immunoglobulin used in the first layer and prepared according to the instructions of the supplier. These FITC antibodies are most often rabbit or goat antibodies.

xii) Rinse four times with PBST.

xiii) View the treated imprints on plastic plates immediately, or mount the slides with cover-slips using glycerol saline at pH 8.5, or a commercially-available mountant.

xiv) Examine under incident ultraviolet (UV) light using a microscope with ×10 eye pieces and ×20 or ×40 objective lenses having numerical aperture of >0.65 and >1.3, respectively. Positive and negative controls must be found to give the expected results prior to any other observation.

4.8. Bioassay

Not available.

4.9. Antibody-~~based~~ or antigen-based detection methods ~~(ELISA, etc.)~~

~~Serological~~ Antibody- or antigen-based methods that detect SVCV must be regarded as presumptive unless fully validated monoclonal or polyclonal antibodies are used, as cross reactions with other ~~viruses~~ closely related sprivivirus (PFRV, GrCRV and TenRV) may occur. Commercially available kits using polyclonal antibodies may lack specificity, and those using monoclonal antibodies may not detect all subgenogroups of SVCV (Dixon & Longshaw, 2005). These techniques should not be used as a screening method.

4.9.1. Antigen enzyme-linked immunosorbent assay (ELISA)

~~*Virus identification by enzyme-linked immunosorbent assay (ELISA)*~~

i) Coat the wells of microplates designed for ELISAs with appropriate dilutions of purified immunoglobulins (Ig) specific for SVCV, in 0.02 M carbonate buffer, pH 9.5 (200 µl well^{-1}). Ig may be polyclonal or monoclonal Ig originating most often from rabbit or mouse, respectively. For the identification of SVCV, monoclonal antibodies (MAbs) specific for certain domains of the nucleocapsid (N) protein are suitable.

ii) Incubate overnight at 4°C.

iii) Rinse four times with PBST.

iv) Block with skim milk (5% in carbonate buffer) or other blocking solution for 1 hour at 37°C (300 µl well^{-1}).

v) Rinse four times with PBST.

vi) Add 2% non-ionic detergent (Triton X-100 or Nonidet P-40) to the virus suspension to be identified.

vii) Dispense 100 µl well^{-1} of two- or four-step dilutions of the virus to be identified, and of the non-infected cell culture harvest (negative control). Also include SVCV positive control virus. Incubate for 1 hour at 37°C.

viii) Rinse four times with PBST.

ix) Add to the wells, 200 µl of horseradish peroxidase (HRPO)-conjugated MAb or polyclonal antibody to SVCV; or polyclonal IgG to SVCV. An MAb to N protein specific for a domain different from the one of the coating MAb and previously conjugated with biotin can also be used. Incubate for 1 hour at 37°C.

x) Rinse four times with PBST.

xi) If HRPO-conjugated antibody has been used, go to step xiii. Otherwise, add 200 µl of HRPO-conjugated streptavidin or ExtrAvidin (Sigma) to those wells that have received the biotin-conjugated antibody and incubate for 1 hour at 37°C.

xii) Rinse four times with PBST.

xiii) Add 200 µl of a suitable substrate and chromogen, such as tetramethylbenzidine dihydrochloride. Stop the course of the test when positive controls react, and read the results.

Enzyme-linked immunosorbent assay (*ELISA*) *using tissue homogenates*

See Section ~~A.~~2.2.2 of Chapter 2.3.0, for obtaining organ homogenates.

i) Coat the wells of microplates designed for ELISAs with appropriate dilutions of purified immunoglobulins (Ig) specific for SVCV, in 0.02 M carbonate buffer, pH 9.5 (200 µl well^{-1}). Ig may be polyclonal or monoclonal Ig originating most often from rabbit or mouse, respectively. For the identification of SVCV, monoclonal antibodies (MAbs) specific for certain domains of the nucleocapsid (N) protein are suitable.

ii) Incubate overnight at 4°C.

iii) Rinse four times with PBST.

iv) Block with skim milk (5% in carbonate buffer) or other blocking solution for 1 hour at 37°C (300 µl well^{-1}).

v) Rinse four times with PBST.

vi) Store a 1/4 aliquot of each homogenate at 4°C, in case the test is negative and virus isolation in cell culture is required.

vii) Treat the remaining part of the homogenate with 2% Triton X-100 or Nonidet P-40 and 2 mM of phenyl methyl sulphonide fluoride; mix gently.

viii) Dispense 100 µl well–1 of two- or four-step dilutions of the sample to be identified, and of negative control tissues. Also include an SVCV positive control virus. Incubate for 1 hour at 37°C.

ix) Rinse four times with PBST.

x) Add to the wells, 200 µl of horseradish peroxidase (HRPO)-conjugated MAb or polyclonal antibody to SVCV; or polyclonal IgG to SVCV. A MAb to N protein specific for a domain different from the one of the coating MAb and previously conjugated with biotin can also be used. Incubate for 1 hour at 37°C.

xi) Rinse four times with PBST.

xii) If HRPO-conjugated antibody has been used, go to step xiv. Otherwise, add 200 µl of HRPO-conjugated streptavidin or ExtrAvidin (Sigma) to those wells that have received the biotin-conjugated antibody and incubate for 1 hour at 37°C.

xiii) Rinse four times with PBST.

xiv) Add 200 µl of a suitable substrate and chromogen, such as tetramethylbenzidine dihydrochloride. Stop the course of the test when positive controls react, and read the results.

xv) If the test is negative, process the organ samples stored at 4°C, for virus isolation in cell culture as described in Section 4.3.

4.9.2. Indirect fluorescent antibody test (IFAT)

~~*Virus identification Confirmation of virus identity by the indirect fluorescent antibody test (IFAT)*~~

i) Prepare monolayers of cells in 2 cm^2 wells of plastic cell culture plates, flasks or on cover-slips or glass slides in order to reach approximately 80% confluency within 24 hours of incubation at 25°C (seed six cell monolayers per virus isolate to be identified, plus two for positive and two for negative controls). The FCS content of the cell culture medium can be reduced to 2–4%. If numerous virus isolates have to be identified, the use of Terasaki plates is strongly recommended.

ii) When the cell monolayers are ready for infection, i.e. on the same day or on the day after seeding, inoculate the virus suspensions to be identified by making tenfold dilution steps directly in the cell culture wells or flasks. For tests using cells cultured on glass cover-slips or slides, the dilutions are made in sterile containers and then used to inoculate the cells.

iii) Dilute the control virus suspension of SVCV in a similar way, in order to obtain a virus titre of about 5000–10,000 PFU ml^{-1} in the cell culture medium.

iv) Incubate at 20°C for 24 hours.

v) Remove the cell culture medium, rinse once with 0.01 M phosphate-buffered saline (PBS), pH 7.2, then three times briefly with cold acetone (stored at –20°C) for slides or cover-slips or 80% acetone in water or 30% acetone in ethanol, also at –20°C, for cells on plastic substrates. Let the fixative act for 15 minutes. A volume of 0.5 ml is adequate for 2 cm^2 of cell monolayer.

vi) Allow the cell monolayers to air-dry for at least 30 minutes and process immediately or freeze at –20°C.

vii) Rehydrate the dried cell monolayers, if they have been stored frozen, by four rinsing steps with ~~PBS containing 0.05% Tween 20~~ PBST and remove this buffer completely after the last rinse. Block with 5% skim milk or 1% bovine serum albumin, in PBST for 30 minutes at 37°C.

viii) Rinse four times with PBST, 5 minutes for each rinse. The slides or plastic culture plates can be gently agitated during the rinses.

ix) Prepare a solution of purified antibody or serum to SVCV in PBST, at the appropriate dilution (which has been established previously or as given by the reagent supplier).

x) Incubate the cell monolayers with the antibody solution for 1 hour at 37°C in a humid chamber and do not allow evaporation to occur.

xi) Rinse four times with PBST.

xii) Incubate the cell monolayers with a solution of fluorescein isothiocyanate (FITC)-conjugated antibody to the immunoglobulin used in the first layer and prepared according to the instructions of the supplier. These FITC antibodies are most often rabbit or goat antibodies.

xiii) Rinse four times with PBST.

xiv) View the treated cell monolayers on plastic substrates immediately, or mount the slides or cover-slips using glycerol saline at pH 8.5, or a commercially available mountant.

xv) Examine under incident ultraviolet (UV) light using a microscope with ×10 eye pieces and ×20 or ×40 objective lenses having numerical apertures of >0.65 and >1.3, respectively. Positive and negative controls must be found to give the expected results prior to any other observation.

4.10. Other ~~serological~~ methods

Not applicable

5. Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations

The method for surveillance of apparently healthy populations ~~susceptible fish~~ ~~populations~~ for declaration of freedom from infection with SVCV is inoculation of cell culture with tissue homogenates ~~extracts~~ (as described in Section 4.3. ~~4.5~~) ~~to demonstrate absence of the virus~~.

This Section only addresses the diagnostic test results for detection of infection in the ~~presence~~ absence (Section 6.1.) or in the presence ~~absence~~ of clinical signs (Section 6.2.) but does not evaluate whether the infectious agent is the cause of the clinical event.

The case definitions for a suspect and confirmed case have been developed to support decision making related to trade and confirmation of disease status at the country, zone or compartment level. Case definitions for disease confirmation in endemically affected areas may be less stringent. It is recommended that all samples that yield suspect positive test results in an otherwise pathogen-free country or zone or compartment should be referred immediately to the OIE Reference Laboratory for confirmation, whether or not clinical signs are associated with the case. If a laboratory does not have the capacity to undertake the necessary diagnostic tests it should seek advice from the appropriate OIE Reference Laboratory.

6.1. Apparently healthy animals or animals of unknown health status[1]

Apparently healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link~~(s)~~ to an infected population. Geographical proximity to, or movement of animals or animal products or equipment~~, etc.,~~ from a known infected population equate to an epidemiological link. Alternatively, healthy populations are sampled in surveys to demonstrate disease freedom.

6.1.1. Definition of suspect case in apparently healthy animals

The presence of infection with SVCV shall be suspected if~~: a positive result has been obtained on at least one animal from~~ at least one of the following ~~diagnostic tests~~ criteria is met:

i) Positive result by conventional RT-PCR ~~a recommended molecular or antigen or antibody detection test~~

ii) SVCV-typical CPE. ~~Cytopathic effect~~ in cell culture ~~(viruses)~~

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with SVCV is considered to ~~shall~~ be confirmed if, in addition to the criteria in Section 6.1.1., ~~positive results has been obtained on at least one animal from two test used in the following combination~~ the following criterion is met:

i) ~~Pathogen isolation AND Conventional~~ SVCV-typical CPE in cell culture followed by virus identification by conventional RT-PCR test ~~followed by~~ and amplicon sequencing.

Reference Laboratories should be contacted for specimen referral when testing laboratories cannot undertake any of the recommended test methods and testing is being undertaken that will result in notification to the OIE.

6.2. Clinically affected animals

Clinical signs are not pathognomonic for infection with SVCV ~~a single disease~~; however they may narrow the range of possible diagnoses. ~~[For many diseases, especially those affecting mollusc, 'clinical signs' are extremely limited and mortality may be the only or most dominant observation.]~~

6.2.1. Definition of suspect case in clinically affected animals

The presence of infection with SVCV shall be suspected if at least one of the following criteria is ~~are~~ met:

[1] For example transboundary *commodities*.

i) Gross pathology or clinical signs associated with the disease as described in this chapter, with or without elevated mortality

ii) Positive result by conventional RT-PCR ~~a recommended molecular or antigen or antibody detection test on at least one animal~~

iii) Positive~~s~~ result by antigen ELISA ~~or IFAT or immunohistochemistry~~

iv) Positive result by IFAT

v) Positive result by immunohistochemistry

vi) SVCV-typical CPE ~~Cytopathic effect~~ in cell culture.

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with SVCV is considered to ~~shall~~ be confirmed if, in addition to the criteria in Section 6.2.1., ~~positive results has been obtained on at least one animal from two test used in the following combination~~ the following criterion is met:

i) ~~Pathogen isolation AND Conventional~~ SVCV-typical CPE in cell culture followed by virus identification by conventional RT-PCR test ~~followed by~~ and amplicon sequencing.

Reference Laboratories should be contacted for specimen referral when testing laboratories cannot undertake any of the recommended test methods and testing is being undertaken that will result in notification to the OIE.

6.3. Diagnostic sensitivity and specificity for diagnostic tests

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with SVCV are provided in Table 6.3. (note: no data are currently available). This information can be used for the design of surveys for infection with SVCV, however, it should be noted that diagnostic performance is specific to the circumstances of each diagnostic accuracy study (including the test purpose, source population, tissue sample types and host species) and diagnostic performance may vary under different conditions. Data is only presented where tests are validated to atleast level two of the validation pathway described in Chapter 1.1.2. and the information is available within published diagnostic accuracy studies.

Table 6.1. *Diagnostic performance of tests recommended for surveillance or diagnosis*

Test type	Test purpose	Source population	Tissue/ sample type	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation
Cell culture	Surveillance, diagnosis	–	Tissue homogenates	–	Not yet available	Not yet available	–	–
RT-PCR	Surveillance, diagnosis	–	Tissue homogenates	–	Not yet available	Not yet available	–	–
RT-PCR	Surveillance, diagnosis	–	Cell culture	–	Not yet available	Not yet available	–	–
~~RT-LAMP*~~	~~Surveillance~~	~~Live imported fish~~	~~Spleen, kidney and brain homogenate~~	~~Common carp, koi, goldfish~~	~~92.6 (27)~~	~~98.2 (445)~~	~~Virus isolation~~	~~Liu *et al.*, 2008~~

DSe = diagnostic sensitivity; DSp = diagnostic specificity; *n* = number of samples used in the study; ~~RT-LAMP: = real-time loop mediated isothermal amplification. *Listed as suitable test~~

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* *

NB: There are OIE Reference Laboratories for Spring viraemia of carp
(see Table at the end of this *Aquatic Manual* or consult the OIE web site for the most up-to-date list:
http://www.oie.int/en/scientific-expertise/reference-laboratories/list-of-laboratories/).
Please contact the OIE Reference Laboratories for any further information on
Spring viraemia of carp

NB: First adopted in 1995 as spring viraemia of carp. Most recent updates adopted in 2012.

CHAPTER 2.1.3.

INFECTION WITH *BATRACHOCHYTRIUM SALAMANDRIVORANS*

1. Scope

Infection with *Batrachochytrium salamandrivorans* ~~(Bsal)~~ means infection of amphibians with the pathogenic agent *Batrachochytrium salamandrivorans*, of the Division Chytridiomycota and Order Rhizophydiales ~~Genus *Batrachochytrium* and Family *Incertae sedis*~~.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

The type strain of the pathogenic chytrid fungal agent *Batrachochytrium salamandrivorans* (Bsal) ~~type strain~~ is AMFP13/1. Three more isolates have been described (Martel *et al*., 2014) but no information is available on genetic structuring or phenotypic variation. Phylogenetic analyses show that Bsal forms a clade with its sister species *B. dendrobatidis* (Martel *et al*., 2013). The genome size of the type strain was determined at 32.6 Mb with 10,138 protein-coding genes predicted (Farrer *et al*., 2017). The contribution of these proteins to virulence is currently not clear.

2.1.2. Survival and stability inside the host tissues ~~in processed or stored samples~~

Bsal is an intracellular pathogen that develops inside epidermal cells. The presence of Bsal could be demonstrated using real-time polymerase chain reaction (~~q~~PCR) on dorsal skin swabs up to 7 days on average post-mortem and using histopathology of dorsal skin tissue up to 3 days on average post-mortem (Thomas *et al*., 2018). It is not clear how long Bsal can survive inside tissues of a dead host and how long a dead host remains infectious. ~~Storage of tissues or skin swabs in 70% ethanol or at –20°C allows detection of Bsal using qPCR for more than 150 years as demonstrated by analysis of museum specimens (Martel *et al*., 2014).~~

2.1.3. Survival and stability outside the host

Encysted spores have been shown to remain infectious in pond water for up to at least 31 days (Stegen *et al*., 2017) and are considered more ~~environmentally~~ resistant in the environment compared with zoospores. Experimentally inoculated forest soil was demonstrated to remain infectious to fire salamanders for 48 hours (Stegen *et al.,* 2017). However, Bsal DNA was detected ~~up to 28 weeks~~ in contaminated forest soil for up to 28 weeks (Stegen *et al*., 2017). ~~However,~~ Whether this reflects the presence of viable Bsal organisms is not clear. The effect of ~~dessication~~ desiccation on Bsal survival has not been studied.

For inactivation methods, see Section 2.4.5.

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with Bsal according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) include: ~~[alpine newt (*Ichthyosaura alpestris*), blue-tailed fire-bellied newt (*Cynops cyanurus*), fire salamander (*Salamandra salamandra*), eastern newt (*Nothophthalmus viridescens*), French cave salamander (*Hydromantes strinatii*), Italian newt (*Lissotriton italicus*), yellow spotted newt (*Neurergus crocatus*), Japanese fire-bellied newt (*Cynops pyrrhogaster*), northern spectacle salamander (*Salamandrina perspicillata*), Tam Dao salamander (*Paramesotriton deloustali*), rough-skinned newt (*Taricha granulosa*), sardinian brook salamander (*Euproctus platycephalus*) and Spanish ribbed newt (*Pleurodeles waltl*)] (under study).~~

Family	Scientific name	Common name
Plethodontidae	*Hydromantes strinatii*	French cave salamander
Salamandridae	*Cynops cyanurus*	blue-tailed fire-bellied newt
	Cynops pyrrhogaster	Japanese fire-bellied newt
	Euproctus platycephalus	sardinian brook salamander
	Ichthyosaura alpestris	Alpine newt
	Lissotriton italicus	Italian newt
	Neurergus crocatus	yellow spotted newt
	Nothophthalmus viridescens	eastern newt
	Paramesotriton deloustali	Tam Dao salamander
	Pleurodeles waltl	Spanish ribbed newt
	Salamandrina perspicillata	northern spectacle salamander
	Salamandra salamandra	fire salamander
	Taricha granulosa	rough-skinned newt

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence for susceptibility according to Chapter 1.5. of the *Aquatic Code* are: [under study]

2.2.3. Non-susceptible species

Species that have been found non-susceptible to infection with Bsal according to Chapter 1.5. of the *Aquatic Code* are: [under study]

2.2.4. Likelihood of infection by species, host life stage, population or sub-populations

Bsal is a pathogenic agent that mainly affects urodeles. Evidence from experimental infections and disease outbreaks in the wild and in captivity show that at least most, if not all, species of the family Salamandridae, as well as species of the family Hynobiidae are likely to become infected when exposed to Bsal. However, differences in susceptibility to infection between species do exist: for example, for fire salamanders ~~(*Salamandra salamandra*)~~, the infectious dose of Bsal was determined to be a theoretical one zoospore, whereas a significantly higher dose was necessary to infect Alpine newts ~~(*Ichthyosaura alpestris*)~~; Stegen *et al*., 2017) and one western Palearctic species (*Lissotriton helveticus*) may be more resistant to infection (Martel *et al*., 2014). For the largest family of salamanders (Plethodontidae), little information is currently available; at least one European species (*Speleomantes strinatii*) can be infected but other, North American species (*Gyrinophilus porphyriticus*, *Plethodon glutinosus,* Ambystomatidae) seem less susceptible to infection (Martel *et al*., 2014). Susceptibility of the family of *Cryptobranchidae* is not clear, with a single infection found in a farmed Chinese giant salamander (*Andrias davidianus*; Zhiyong *et al*., 2018). No information is available on the urodele families *Proteidae, Rhyacotritonidae* and *Amphiumidae*. Bsal infection in anurans has only been detected in two species, in captivity, the wild and in lab trials (Nguyen *et al*., 2017; Stegen *et al*., 2017).

Thus far, infections with Bsal have been demonstrated only in amphibians post-metamorphosis. In one experimental infection trial, larvae of fire salamanders were exposed to Bsal, but did not become ~~were not~~ infected (Van Rooij *et al*., 2015). The extent to which factors such as ~~like~~ age and sex affect susceptibility to infection post-metamorphosis is unknown.

In Europe, Bsal has been detected in captive collections of urodeles (Fitzpatrick *et al*., 2018, Sabino-Pinto *et al*., 2015) and the pet trade in salamanders and newts has been hypothesised to play a central role in the distribution of this fungus (Fitzpatrick *et al*., 2018; Yap *et al*., 2015; Zhiyong *et al*., 2018). Hence, urodeles that come into contact with traded urodeles, either directly (~~by~~ via co-housing or contact ~~of~~ with wild animals ~~with~~ or released or captive animals) or indirectly (via materials, contaminated water or soil) ~~come in contact with traded urodeles~~, may have a high likelihood of exposure to infection with ~~be more likely to contract~~ Bsal ~~infection~~.

2.2.5. Distribution of the pathogen in the host

Bsal only infects the skin, where it remains limited to the epidermis.

2.2.6. Aquatic animal reservoirs of ~~Persistent~~ infection

A large number of salamanders, mainly belonging to the families Salamandridae and Hynobiidae, may survive episodes of infection (for example Alpine newts) or be considered tolerant, resulting in persistent subclinical infections. Although persistent infection has not been demonstrated for all species, in the native Bsal range in east Asia, Bsal infection and disease dynamics appear to be consistent for all species examined and appear capable of long-term persistent infections (Laking *et al*., 2017; Martel *et al*., 2014; Zhiyong *et al*., 2018).

In its invasive range, persistent infections (e.g. in Alpine newts) have been implicated in the ~~extirpation~~ local extinction of a highly susceptible species (fire salamanders). It is currently not clear which of the species, mentioned in Section 2.2.1 may sustain persistent infections in the invasive Bsal range. At least some species (the best-known example is the fire salamander) are highly susceptible and invariably die shortly ~~briefly~~ after exposure (Martel *et al*., 2014; Stegen *et al*., 2017), making ~~which would make~~ them unlikely to sustain persistent infections.

It is not known whether other, biotic reservoirs of Bsal exist.

2.2.7. Vectors

There is evidence that birds may carry zoospores attached to their ~~the~~ feet ~~of birds~~ (Stegen *et al*., 2017)~~, which may~~ and thus may act as vectors for Bsal.

2.3. Disease pattern

2.3.1. Mortality, morbidity and prevalence

In its native range in east Asia, Bsal has been demonstrated to be present in the wild at a prevalence of between 2 and 4% on average (data from China [People's Rep. of], Japan, Thailand, and Vietnam; Laking *et al*., 2017; Martel *et al*., 2014; Zhiyong *et al*., 2018), but in the absence of any observed morbidity or mortality under natural conditions. In some populations (*Paramesotriton hongkongensis*), prevalence may reach 50% (Zhiyong *et al*., 2018). In its invasive range in Europe, Bsal was present in a population of fire salamanders at a prevalence of between 25 and 63% (Stegen *et al*., 2017). ~~In captive collections of urodeles in Europe,~~ Bsal occurrence and associated mortality has been ~~were~~ detected in captive collections of urodeles in Europe, including Germany (1), the United Kingdom (4), Belgium (1), the Netherlands (2) and Spain (1) (number in brackets indicates number of collections). When left untreated, morbidity and mortality can reach 100%, at least in members of the genus *Salamandra*.

Morbidity, mortality and minimum infectious dose vary considerably between species (Martel *et al*., 2014; Stegen *et al*., 2017). Based on natural outbreaks in captivity and in the wild and in ~~on~~ infection trials, the case morbidity and case mortality rate in fire salamanders can reach 100%, independent of the initial level of Bsal exposure. This has resulted in the loss of over 99.9% of the fire salamander population at the Bsal index outbreak site in the Netherlands (Spitzen-van der Sluijs *et al*., 2016). All tested western Palearctic urodeles, except for *Lissotriton helveticus* and *Salamandrella keyserlingii*, showed 100% morbidity and mortality when exposed to a single, high dose of Bsal (Martel *et al*., 2014). However, at least for Alpine newts, the case morbidity and case fatality rates depend on the Bsal dose that the animal is exposed to: a high dose resulting in the highest mortality, while a low dose does not necessarily result in morbidity or mortality.

~~It is important to mention that~~ Morbidity and mortality also depend on environmental temperature. For the Bsal type strain, temperatures above 20°C reduces the level of ~~tempers~~ infection and temperatures above 25°C eventually result in killing of Bsal and elimination of infection (Blooi *et al*., 2015b ~~2015a~~). Exposure of infected animals to conditions that inhibit Bsal growth may thus result in non-clinical or sub-clinical infections in susceptible species.

2.3.2. Clinical signs, including behavioural changes

Chytridiomycosis caused by Bsal may be accompanied by a combination of the following signs: epidermal ulcerations (ranging from discrete ~~tiny~~ to extensive), excessive skin shedding, skin haemorrhages and/or fluid loss, anorexia, apathy, abnormal body postures and convulsions ~~and death~~ (Martel *et al.,* 2013).

2.3.3 Gross pathology

Skin anomalies (haemorrhages, ulcerations, presence of sloughed skin) are the main pathological findings (Martel *et al.,* 2013).

2.3.4. Modes of transmission and life cycle

Colonial or monocentric thalli of this fungus develop inside host epidermal cells and produce motile zoospores or walled, encysted spores, both of which are infectious stages. Zoospores are released through one or several discharge tubes. While motile spores actively swim towards a suitable substrate (e.g. a host), the encysted spores float at the water–air interface and passively adhere to a passing host (Stegen *et al.,* 2017). *In vitro*, developing thalli form fine rhizoids. Mature thalli *in vitro* are between 16 and 50 µm in diameter, *in vivo* between 7 and 17 µm; zoospores are approximately 5 µm in diameter. Motile zoospores are roughly spherical, the nucleus is located outside of the ribosomal mass, with aggregated ribosomes, multiple mitochondria and numerous lipid globules. The position of the non-flagellated centriole in free swimming zoospores varies from angled to parallel to the kinetosome (Martel *et al.,* 2013).

There are no indications of vertical transmission. However, this cannot be excluded in species giving birth to metamorphosed offspring (e.g. *Salamandra atra*, *Salamandra lanzai*, *Lyciasalamandra helverseni*). Horizontal transmission occurs through direct contact or contact with contaminated soil or water (Stegen *et al*., 2017). Infectious stages include the motile zoospore and the environmentally resistant encysted spores (Stegen *et al*., 2017). Infections can be reproduced under experimental conditions by topically applying a Bsal inoculum on the dorsum of amphibians and housing the exposed animals at 15°C (Martel *et al*., 2013; 2014; Stegen *et al*., 2017). This inoculum can either contain motile zoospores or the immobile, encysted spores.

Pathways of Bsal dispersal within Europe are poorly understood but may be anthropogenic (e.g. through contaminated material). Zoospores attach to bird feet, suggesting birds may spread Bsal over larger distances (Stegen *et al*., 2017). Direct animal-to-animal contact is necessary for transmission of Bsal: salamanders only separated by 1 cm from infected conspecifics were not infected in laboratory trials, in contrast to co-housed animals (Spitzen-van der Sluijs *et al*., 2018). Overall, dispersal ability of Bsal in Europe currently seems limited: Bsal was found not to be transmitted to a neighbouring site in the Netherlands, despite being downstream of a small stream, and the current distribution of Bsal in Europe is probably not continuous (Spitzen-van der Sluijs *et al*., 2018).

Although Bsal dispersal between populations is now hypothesised to be mainly human mediated, other factors (e.g. wildlife, water) may play key roles and critical knowledge about Bsal dispersal is currently lacking.

2.3.5. Environmental and management factors

The Bsal type strain AMFP13/1 tolerates temperatures up to 25°C but is killed at higher temperatures (Blooi *et al*., 2015b ~~2015a~~). As Bsal infections have been demonstrated in aquatic newts at water temperatures above 25°C (Laking *et al*., 2017; Zhiyong *et al*., 2018), it is likely~~, however,~~ that thermal tolerance may be Bsal lineage dependent. A temperature of 4°C results in slower progression ~~build-up~~ of infection but does not reduce morbidity or mortality (Stegen *et al*., 2017). Desiccation is likely to be poorly tolerated by Bsal, although data are currently lacking, and the encysted spore may be resistant to drying (Stegen *et al*., 2017; Van Rooij *et al*., 2015). It is not known to what extent Bsal tolerates freezing.

Co-occurrence of highly susceptible species such as fire salamanders with less susceptible species, such as Alpine newts may facilitate density independent disease dynamics that lead to the ~~extirpation~~ local extinction of the highly susceptible species (Stegen *et al*., 2017).

Barriers to pathogen dispersal, for example those preventing migration of infected hosts such as amphibian fences or roads, or those preventing transmission by potential Bsal vectors including humans, fomites and wildlife, may prevent transmission at small spatial scales (Spitzen-van der Sluijs *et al*., 2018).

2.3.6. Geographical distribution

Asia is currently considered the region of origin of Bsal (Martel *et al*., 2014), where the infection appears to be endemic in amphibian communities across a wide taxonomic, geographical and environmental range, albeit at a low prevalence between 2 and 4% (Zhiyong *et al.,* 2018). In Asia, Bsal was shown to be widely present in urodele populations in China (People's Rep. of), Japan, Thailand and Vietnam. East Asia is presumed to be the native range of the fungus (Laking *et al*., 2017; Martel *et al*., 2014; Zhiyong *et al.,* 2018).

Europe is considered the invasive range of the fungus where Bsal was first identified during a mortality event in fire salamanders ~~(*Salamandra salamandra*)~~ in Bunderbos, the Netherlands (Martel *et al.,* 2013). In Europe, Bsal was detected by surveys of wild susceptible species in Belgium, Germany and the Netherlands (Martel *et al.*, 2014; Spitzen-van der Sluijs *et al.,* 2016), and in captive urodele populations in Belgium, Germany, the Netherlands, Spain, and the United Kingdom (Fitzpatrick *et al.,* 2018; Sabino-Pinto *et al.,* 2015).

Bsal has not been reported in Africa or the Americas.

2.4. Biosecurity and disease control strategies

2.4.1. Vaccination

Not available.

2.4.2. Chemotherapy including blocking agents

A combined treatment using Polymyxin E, voriconazole and a temperature regime of 20°C has been shown to be effective in eradicating Bsal from infected hosts (Blooi *et al*., 2015c ~~2015b~~). If the treatment is not performed properly and does not achieve eradication, low level carriers are created and the likelihood of Bsal detection, is reduced.

2.4.3. Immunostimulation

Not available.

2.4.4. Breeding resistant strains

~~Breeding resistant strains is one of the few options for long term sustainable disease mitigation.~~

No information available.

2.4.5. Inactivation methods

Bsal is sensitive to a wide variety of disinfectants (Van Rooij *et al*., 2015). Inactivation using formalin has been shown to hamper DNA detection using real-time PCR ~~qPCR~~. Bsal is killed within 30 seconds in 70% ethanol (Van Rooij *et al.,* 2017). Inactivation in 70% ethanol allows for subsequent molecular tests yet is less suitable for histopathology. The Bsal type strain AMFP 13/1 is killed at temperatures exceeding 25°C; consequently, inactivation of this fungus can be achieved through heat treatment by autoclaving (Martel *et al.*, 2013).

2.4.6. Disinfection of eggs and larvae

No information available.

2.4.7. General husbandry

In captivity, pathogen detection is difficult due to low prevalence in subclinically infected animals that often carry Bsal at low intensities (Martel *et al*., 2014; Zhiyong *et al*., 2018). These subclinically infected animals often belong to (but are not restricted to) taxa of Asian urodeles. Highly susceptible species (such as fire salamanders ~~*Salamandra salamandra*~~) may serve a sentinel function. Temperature regimes in captivity may strongly interfere with pathogen detection. Temperatures higher than 20°C (and below 25°C) severely impair pathogen proliferation in the host skin (Blooi *et al*., 2015b ~~2015a~~) and may result in infections that cannot be detected.

Heat treatment can be used to clear infection with Bsal in thermotolerant salamander species (Blooi *et al*., 2015a).

3. Specimen selection, sample collection, transportation and handling

This Section draws on information from Sections 2.2., 2.3. and 2.4. to identify populations, individuals and samples which are most likely to be infected.

3.1. Selection of populations and individual specimens

In case of disease or mortality in urodeles in captivity, sampling should be focused primarily on diseased or moribund animals (i.e. those showing skin lesions and abnormal behaviour). In a population with ongoing disease and mortality, live but diseased animals are preferentially sampled. The second choice is dead animals. Only freshly dead animals should be sampled as detectability of Bsal deteriorates post-mortem (Thomas *et al*., 2018). However, in the absence of diseased or freshly dead animals, apparently healthy animals can be sampled.

Similarly, in wild populations, samples should be taken preferentially from diseased ~~or~~, moribund or freshly dead animals ~~should preferentially be sampled, but~~; however, as these may quickly be removed (i.e. through predation, scavenging) only healthy animals may ~~only~~ be available. Populations which have declined or where dead animals have been observed should be targeted.

3.2. Selection of organs or tissues

The only relevant tissue is skin tissue and probably only from amphibians post-metamorphosis. Both invasive (skin biopsies) and non-invasive (cotton tipped swabs) samples ~~sampling~~ are appropriate, given the apical shedding of Bsal spores. In dead animals, dorsal skin is the preferred tissue, given its slower post-mortem decay (Thomas *et al*., 2018).

3.3. Samples or tissues not suitable for pathogen detection

~~Any other t~~Tissues other than skin ~~is~~ are not suitable for the detection of Bsal in amphibians.

3.4. Non-lethal sampling

Non-lethal sampling is possible, either by collecting skin biopsies (toeclips or tailclips) or by non-invasively collecting samples using cotton tipped swabs. The latter is preferred given its minimal impact on animal welfare ~~well-being~~. As Bsal is limited to the superficial skin layers of the amphibian host, non-lethal sampling results are equivalent to lethal sampling results. ~~In the absence of other, Bsal specific diagnostic tests (other than the laborious isolation of the fungus),~~ Large numbers of animals can be sampled using skin swabs with minimal effects on animal welfare. Cotton tipped swabs should be rubbed firmly over the abdomen (10 times), the underside of a foot (10 times) and the ventral tail (10 times) using the tip of the swab. The use of disposable gloves for manipulating amphibians is highly recommended.

3.5. Preservation of samples for submission

3.5.1. Samples for pathogen isolation

Bsal isolation is a ~~very~~ laborious procedure, requiring up to two months to obtain ~~for obtaining~~ a pure culture from a clinical sample. Isolation from animals that died due to Bsal infection is hampered by bacterial overgrowth. The best sample for Bsal isolation is a diseased, living animal, which is euthanised just prior to an isolation attempt. Before sampling diseased animals should be kept at temperatures between 5 and 15°C to avoid clearance of infection (Blooi *et al*., 2015b ~~2015a~~).

3.5.2. Preservation of ~~Fixed~~ samples for molecular detection

Tissue samples for PCR testing should be preserved in 70–90% (v/v) analytical/reagent-grade (undenatured) ethanol. The recommended ratio of ethanol to tissue is 10:1. The use of lower grade (laboratory or industrial grade) ethanol is not recommended. If material cannot be fixed it may be frozen.

Skin swabs should be stored dry and preferably frozen.

3.5.3. ~~Fixed~~ Preservation of samples for histopathology, immunohistochemistry or *in-situ* hybridisation

Skin samples for histopathology should be fixed immediately after collection. The recommended ratio of formalin (10%) to tissue is 10:1.

3.5.4. ~~Fixed~~ Preservation of samples for electron microscopy

For transmission electron microscopy, skin samples can be fixed in glutaraldehyde in 0.05 M sodium cacodylate buffer and 1% osmium tetroxide post-fixation (Martel *et al*., 2013).

3.5.5. Samples for other tests

Not applicable.

3.6. Pooling of samples

Pooling of up to four ~~five~~ skin swab samples appears to allow reliable detection of Bsal in clinically affected animals (Sabino-Pinto *et al*., ~~2018~~ 2019a; 2019b) but estimates of ~~on~~ the impact on diagnostic performance of the test ~~characteristics~~ have not been determined. Given low infection intensities in subclinically infected animals, sampling and testing of individual animals is recommended.

4. Diagnostic methods

The methods currently available for identifying infection that can be used in i) surveillance of apparently healthy populations), ii) presumptive and iii) confirmatory diagnostic purposes are listed in Table 4.1. by life stage. The designations used in the Table indicate:

Key:

+++ =	Recommended method(s) validated for the purpose shown and usually to stage 3 of the OIE Validation Pathway;
++ =	Suitable method(s) but may need further validation;
+ =	May be used in some situations, but cost, reliability, lack of validation or other factors severely limits its application;
Shaded boxes =	Not appropriate for this purpose.

The selection of a test for a given purpose depends on the analytical and diagnostic sensitivities and specificities repeatability and reproducibility. OIE Reference Laboratories welcome feedback on diagnostic performance for assays, in particular PCR methods, for factors affecting assay analytical sensitivity or analytical specificity, such as tissue components inhibiting amplification, presence of nonspecific or uncertain bands, etc., and any assays that are in the +++ category.

Table 4.1. OIE recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals

Method [amend or delete as relevant]	A. Surveillance of apparently healthy animals				B. Presumptive diagnosis of clinically affected animals				C. 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
Wet mounts	+	+	+	1	+	+	+	1				~~1~~
Histopathology[3]	+	+	+	1	++	++	++	1				~~1~~
Cell or artificial media culture									+	+	+	1
Real-time PCR	+++	+++	+++	2	+++	+++	+++	2	+++	+++	+++	2
Conventional PCR												
Amplicon sequencing[4]												
In-situ hybridisation												
LAMP												
Lateral flow assay	~~+~~	~~+~~	~~+~~	~~1~~	+~~+~~	+~~+~~	+~~+~~	1				~~1~~
Immunohistochemistry												

LV = level of validation, refers to the stage of validation in the OIE Pathway (Chapter 1.1.2.); PCR = polymerase chain reaction; LAMP = loop-mediated isothermal amplification.
[1]For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). [2]Early and juvenile life stages have been defined in Section 2.2.4.
[3]Cytopathology and histopathology can be validated if the results from different operators has been statistically compared. [4]Sequencing of the PCR product.
Shading indicates the test is inappropriate or should not be used for this purpose.

4.1. Wet mounts

Wet mounts of skin scraping or pieces of shed skin can be examined at magnification 10× using light microscopy. The presence of motile spores of approximately 5 µm are indicative of amphibian chytrid infection.

4.2. Histo- and cytopathology

~~No reports are available on the use of cytology.~~ Histopathology of skin in amphibian post-metamorphosis may provide strong indications of Bsal infection. In ~~a~~ haematoxylin/eosin ~~staining of skin~~ stained sections, histopathological evidence suggestive of Bsal infections of skin, ~~is~~ multifocal epidermal necrosis with loss of distinction between layers of keratinocytes associated with myriad intracellular and extracellular chytrid-type fungal thalli provides histopathological evidence of Bsal infection (Martel *et al*., 2013; White *et al*., 2016). Using immunohistochemistry, Bsal thalli can be stained, which aids in detecting low level infections (Thomas *et al*., 2018). Histopathology is highly indicative, yet does not allow ~~specific~~ definitive identification of Bsal, which needs further confirmation. In randomly collected skin samples from experimentally infected salamanders, histopathology was capable of detecting Bsal in only a minority of the samples (Thomas *et al*., 2018). In dead animals, post-mortem decay of the epidermis may mask the lesions (Thomas *et al*., 2018). Lesions can be so extensive, that the epidermis is entirely eroded and no fungal thalli can be observed. Mild infections can be missed due to the multifocal and small lesions (Thomas *et al*., 2018). ~~For asymptomatically~~ In subclinically infected animals, sensitivity should be rated low. ~~Sensitivity,~~ In clinically affected animals, sensitivity and specificity of histopathology and immunohistochemistry have not been quantified.

No reports are available on the use of cytopathology.

4.3. Cell or artificial media culture for isolation

Bsal can be isolated and cultured on artificial media, yet this is a laborious and difficult procedure, typically requiring between 4 weeks and 2 months. There is a significant probability of bacterial overgrowth, which hampers fungal isolation, resulting in poor sensitivity. The protocol of Fisher *et al*. (2018) can be used. Small (approximately 1 mm^2) pieces of skin from an infected, diseased animal should first be thoroughly cleaned by wiping through agar plates. The cleaned pieces of skin can then each be transferred to a well of a 96-well plate, containing tryptone-gelatin hydrolysate lactose broth (TGhL) containing penicillin/streptomycin (200 mg/litre) and incubated at 15°C. Wells showing chytrid growth without bacterial contamination can be used for subculturing (Martel *et al*., 2013). Chytrid growth can be visualised by examining the wells under an inverted microscope (10–40 × magnification).

Given the difficulties to isolate Bsal from infected animals and the high uncertainty to obtain a viable culture, this method is not appropriate as ~~first diagnostic approach~~ a routine diagnostic method, but ~~(~~in rare cases~~)~~ may be useful to confirm infection ~~and for~~ or to obtain~~ing~~ isolates for research ~~(for example for epidemiological tracing)~~.

4.4. Nucleic acid amplification

4.4.1. Real-time PCR

The following information is derived from Blooi *et al*. (2013), Thomas *et al*. (2018) and Sabino Pinto *et al*. (2018). DNA from skin swabs can be extracted using commercial DNA extraction kits. ~~in 100 µl Prepman Ultra Reagent (Applied Biosystems, Foster City, CA) or by using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). The latter follows the animal tissues protocol (Qiagen DNeasy Blood and Tissue kit) with pre-treatment for Gram-positive bacteria and expanded initial incubation for 1 hour. DNA from skin tissue can be extracted using proteinase K digestion or DNA Easy Tissue Kit.~~ Extracted DNA is diluted tenfold to minimise possible PCR inhibition. Controls should be run with each assay: at least a negative extraction control and a positive control; preferably, an internal PCR control is included. Positive control consists of DNA extracts of a tenfold dilution series of Bsal zoospores from 1 to 100.000 to allow quantification.

A TaqMan PCR has been partially validated to level 2 without however, stating its intended purpose (Thomas *et al*., 2018). SYBR green real-time PCR, may be used as well but needs further validation to determine specificity and sensitivity (Martel *et al*., 2013). The TaqMan PCR can either be used as simplex PCR or in combination with primers to detect *B. dendrobatidis* in a duplex PCR (Blooi *et al*., 2013) and uses the forward primer STerF (5'-TGC-TCC-ATC-TCC-CCC-TCT-TCA-3'), reverse primer STerR (5'-TGA-ACG-CAC-ATT-GCA-CTC-TAC-3') and Cy5 labelled probe STerC (5'-ACA-AGA-AAA-TAC-TAT-TGA-TTC-TCA-AAC-AGG-CA-3') to detect the presence of the 5.8S rRNA gene of Bsal. Intra- and interassay efficiency were 94 and 99%, respectively (Blooi *et al*., 2013). This

TaqMan duplex PCR does not decrease detectability of both Bd and Bsal, except in case of mixed infections (Thomas *et al*., 2018). The use of simplex Bsal-specific PCR is therefore recommended in case Bd has been detected in the sample. The sensitivity of this real-time ~~q~~PCR is between 96 and 100% and diagnostic specificity 100% (95% CI: 73–100%; Thomas *et al*., 2018) when used in clinically affected animals. Although DNA quantities as low as 0.1 genomic equivalent can be detected (Blooi *et al*., 2013), Thomas *et al*. (2018) recommend a threshold of 1 genomic equivalent per reaction to reduce the likelihood of false positive results. Borderline results (≤ 1 GE per reaction) should be classified as suspect and need confirmation by sequencing (or isolation).

Samples are preferably run in duplicate. A sample is considered positive based on the combination of (1) the shape of the amplification curves (2) positive results in both duplications, (3) returning GE values above the detection threshold (1 GE per reaction) (4) low variability between duplicates (< 0.3 Ct value).

4.4.2. Conventional PCR (PCR)

~~The use of real-time PCR is recommended.~~ No conventional PCR protocol has been validated.

4.4.3. Other nucleic acid amplification methods

None validated.

4.5. Amplicon sequencing

~~For confirmation of suspect samples, amplified products can be sequenced with the primers as described in 4.4.1.~~

No conventional PCR protocol has been validated.

4.6. *In-situ* hybridisation ~~(and histoimmunochemistry)~~

No *In-situ* hybridisation~~:~~ ~~no~~ validated protocols are available.

4.7. Immunohistochemistry

Immunohistochemistry is currently not Bsal specific, due to the lack of Bsal specific antibodies (Dillon *et al*., 2017; Thomas *et al*., 2018). ~~Sensitivity of immunohistochemistry in diseased or dead animals can be estimated to be high if clinically affected skin regions have been selected.~~

4.8. Bioassay

Not available.

4.9. Antibody-based or antigen detection methods

A lateral flow assay (LFA) using an IgM monoclonal antibody (MAb) was developed to detect infection in amphibian skin samples. This MAb does not discriminate between *B. salamandrivorans, B. dendrobatidis* and *Homolaphlyctis polyrhiza* (Dillon *et al.*, 2017 ~~2016~~). The sensitivity of this test is likely to be lower than that of the real-time ~~q~~PCR (Dillon *et al.*, 2017): in experimentally Bd inoculated frogs, 1/5 animals tested positive in LFA compared to 4/5 using real-time ~~q~~PCR. This would make this technique most useful in animals with high infection loads. Such techniques may be useful for point-of-care testing if specificity is increased and provided thorough validation.

4.10. Other ~~serological~~ methods

Not applicable

5. Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations

The use of real-time PCR on skin swabs is recommended for surveillance.

6. Corroborative diagnostic criteria

This Section only addresses the diagnostic test results for detection of infection in the ~~presence~~ absence (Section 6.1.) or in the presence ~~absence~~ of clinical signs (Section 6.2.) but does not evaluate whether the infectious agent is the cause of the clinical event.

The case definitions for a suspect and confirmed case have been developed to support decision making related to trade and confirmation of disease status at the country, zone or compartment level. Case definitions for disease confirmation in endemically affected areas may be less stringent.

6.1. Apparently healthy animals or animals of unknown health status[2]

Apparently healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link(s) to an infected population. Geographic proximity to, or movement of animals or animal products or equipment, etc., from a known infected population equate to an epidemiological link. Alternatively, healthy populations are sampled in surveys to demonstrate disease freedom.

Such surveys typically consist of non-invasive sampling using skin swabs that are examined for the presence of Bsal using real-time PCR. When applied to animals in the wild, confirmation by using a complementary technique, other than sequencing the PCR product, is often not feasible.

6.1.1. Definition of suspect case in apparently healthy animals

The presence of infection with Bsal shall be suspected if ~~a positive result has been obtained on at least one animal from at least~~ one of the following ~~diagnostic tests~~ criteria is met:

i) Positive result by real-time PCR.

ii) Histopathological changes ~~(including immunohistochemistry)~~ consistent with the presence of the pathogen or the disease.

iii) The presence of motile spores, compatible with chytrid zoospores, in wet mount of urodele skin.

~~iv) Positive result from lateral flow assay (LFA).~~

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with Bsal is confirmed if ~~positive results have been obtained on at least~~, in addition to the criteria in Section 6.1.1., one ~~animal from two tests used in~~ of the following ~~combination~~ criteria is met:

i) Positive result by real-time PCR. ~~on skin swab or skin tissue, and by histopathology or immunohistochemistry on skin tissue~~.

ii) ~~Positive result by real-time PCR on skin swab or skin tissue, and~~ Pathogenic agent isolation from the skin in culture and ~~confirmation~~ identification by real-time PCR.

[2] For example transboundary *commodities*.

6.2. Clinically affected animals

Clinical signs are not pathognomonic for a single disease; however, they may narrow the range of possible diagnoses.

6.2.1. Definition of suspect case in clinically affected animals

The presence of infection with Bsal shall be suspected if at least one of the following criteria is met:

i) Clinical signs (haemorrhages, ulcerations, presence of sloughed skin, see Section 2.3.2.), notably the presence of skin ulcers and/or disecdysis.

ii) Positive result by real-time PCR. ~~on at least one swab or skin tissue~~.

iii) Histopathological changes consistent with the presence of the pathogenic agent or the disease.

iv) Visual observation (by microscopy) of motile spores, compatible with amphibian chytrid zoospores, in a wet mount of the skin of at least one diseased urodele.

v) Positive result of antigen detection ~~technique such as~~ by LFA.

~~vi) Positive result from immunohistochemistry.~~

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with Bsal is confirmed if, in addition to the criteria in Section 6.2.1., ~~positive results have been obtained on at least one animal from~~ ~~two tests used in~~ one of the following ~~combination diagnostic tests~~ criteria is met:

i) Positive result by real-time PCR. ~~on skin swab or skin tissue and by histopathology~~.

ii) ~~Positive result by real-time PCR on skin swab or skin tissue, and~~ Pathogenic agent isolation from the skin in culture and identification by real-time PCR ~~and confirmation by real-time PCR~~.

~~Reference Laboratories should be contacted for specimen referral when testing laboratories cannot undertake any of the recommended test methods and testing is being undertaken that will result in notification to the OIE.~~

6.3. Diagnostic sensitivity and specificity for diagnostic tests

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with Bsal are provided in Table 6.3. This information can be used for the design of surveys for infection with Bsal, however, it should be noted that diagnostic performance is specific to the circumstances of each diagnostic accuracy study (including the test purpose, source population, tissue sample types and host species) and diagnostic performance may vary under different conditions. Data is only presented where tests are validated to at least level two of the validation pathway described in Chapter 1.1.2. and the information is available within published diagnostic accuracy studies.

Table 6.1. Diagnostic performance of tests recommended for surveillance or diagnosis

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation
Real-time PCR	Diagnosis	Experimentally infected salamanders (clinical and subclinical infection)	Skin swabs	*Salamandra salamandra*	96–100 (26)	100 (12)	Droplet digital PCR	Thomas *et al.* (2018)

DSe = diagnostic sensitivity; DSp = diagnostic specificity; *n* = number of samples used in the study.

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NB: There are currently no OIE Reference Laboratories for infection with *Batrachochytrium salamandrivorans*
NB: First adopted in 2020.

CHAPTER 2.3.4.

INFECTION WITH INFECTIOUS HAEMATOPOIETIC NECROSIS VIRUS

1. Scope

Infection with infectious haematopoietic necrosis virus means infection with the pathogenic agent Salmonid novirhabdovirus (commonly known as infectious haematopoietic necrosis virus [IHNV]) of the Genus *Novirhabdovirus* and Family *Rhabdoviridae*.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

IHNV consists of a bullet-shaped particle of approximately 150–190 nm in length and 65–75 nm in diameter that encapsulates a non-segmented, negative-sense, single-stranded RNA genome of approximately 11,000 nucleotides. The viral genome codes for six proteins in the following order: a nucleoprotein (N), a phosphoprotein (P), a matrix protein (M), a glycoprotein (G), a non-virion protein (NV), and a polymerase (L). Due to the primary position of the nucleoprotein gene on the IHNV genome, nucleoprotein transcripts and protein are the first and most abundant during viral infection and is typically the preferred target of diagnostic tests. The glycoprotein forms spike-like projections on the surface of the mature virion and is the primary antigenic component of the virus such that anti-glycoprotein serum is sufficient to neutralise infections.

The type strain of IHNV is the Western Regional Aquaculture Center (WRAC) strain available from the American Type Culture Collection (ATCC VR-1392). The GenBank accession number of the genomic sequence of the WRAC strain is L40883 (Morzunov *et al.,* 1995; Winton & Einer-Jensen, 2002).

Phylogenetic analyses based on G-gene nucleotide sequences have classified IHNV isolates into five major genogroups denoted U, M, L, E, and J that correspond to geographical location rather than host species (Cieslak *et al.,* 2017; Enzmann *et al.,* 2005; 2010; Johansson *et al.,* 2009; Kim *et al.,* 1999; Kolodziejek *et al.,* 2008; Kurath *et al.,* 2003; Nishizawa *et al.,* 2006). Nevertheless, IHNV displays a strong phylogeographic signature reflecting the host species from which the virus is most commonly isolated in various geographical areas (e.g. sockeye salmon [*Oncorhynchus nerka*] in the Northeast Pacific – U genogroup; Chinook salmon [*Oncorhynchus tshawytscha*] in California, USA – L genogroup; and rainbow trout [*Oncorhynchus mykiss*] in Europe, Asia, and Africa (Mulei *et al.,* 2019) and ~~Idaho,~~ USA – E, J and M genogroups, respectively). Additionally, experimental infections demonstrating that U and M genogroup viruses had higher virulence in sockeye salmon and rainbow trout, respectively (Garver *et al*., 2006), supports the observation that IHNV strains isolated from its historical phylogeographic host tends to be more virulent for the same species in comparison to other species.

2.1.2. Survival and stability in processed or stored samples

IHNV stability in host tissues during storage and processing is largely influenced by temperature. The virus is more stable at lower temperature and remained infectious for at least 3 days at 4°C in naturally infected or IHNV-seeded tissue (Burke & Mulcahy, 1983; Gosting & Gould, 1981; Hostnik *et al.,* 2002; Pietsch *et al.*, 1977). For long-term survival of infectious virus, tissues should be stored at temperatures below –20°C (Burke & Mulcahy, 1983; McClure *et al.,* 2008). The preferred method for retaining infectious virus is to maintain the IHNV sample on ice with rapid processing and inoculation of cell cultures as soon as possible due to the progressive reduction in titre with increasing temperature (Barlic-Maganja *et al.,* 2002; Gosting & Gould, 1981).

2.1.3. Survival and stability outside the host

IHNV can survive outside the host tissue in fresh water and sea water, but is ~~impacted~~ affected by temperature, ultraviolet (UV) exposure, microbial community and suspended sediments. ~~At 4°C–15°C, 10^5 pfu/ml of IHNV remained detectable via cell culture after 1 week in either fresh or salt water (Kell *et al.,* 2014).~~ For all genotypes, inactivation rates are reduced at lower water temperatures and virions remain infectious for longer in freshwater compared with seawater (Kell *et al.,* 2014). However, when exposed to sunlight (UV-A and UV-B), IHNV at the water surface is rapidly inactivated with six orders of magnitude of virus rendered non-infectious within 3 hours (Garver *et al.,* 2013). In addition, infectious virus is inactivated by the microbial community within the water source and with increased amounts of suspended sediments (Garver *et al.,* 2013; Kamei *et al.,* 1987).

For inactivation methods, see Section 2.4.6.

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with IHNV according to Chapter 1.5 of *Aquatic Animal Health Code* (*Aquatic Code*) are:

Family	Scientific name	Common name
Esocidae	*Esox lucius*	Northern pike
Salmonidae	*Salmo marmoratus*	Marble trout
	Salmo salar	Atlantic salmon
	Salmo trutta	Brown trout
	Salvelinus alpinus	Arctic char
	Salvelinus fontinalis	Brook trout
	Salvelinus namaycush	Lake trout
	Oncorhynchus clarki	Cutthroat trout
	Oncorhynchus tshawytscha	Chinook salmon
	Oncorhynchus keta	Chum salmon
	Oncorhynchus kisutch	Coho salmon
	Oncorhynchus masou	Mas~~o~~u salmon
	Oncorhynchus mykiss	Rainbow trout
	Oncorhynchus nerka	Sockeye salmon

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to infection with IHNV according to Chapter 1.5 of the *Aquatic Code* are: White sturgeon (*Acipenser transmontanus*), European eel (*Anguilla anguilla*), Tube-snout (*Aulorhynchus flavidus*), Pacific herring (*Clupea pallasii*), Shiner perch (*Cymatogaster aggregate*) and Turbot (*Scophthalmus maxima*).

~~Family~~	~~Scientific name~~	~~Common name~~
~~Acipenseridae~~	~~*Acipenser transmontanus*~~	~~White sturgeon~~
~~Anguillidae~~	~~*Anguilla anguilla*~~	~~European eel~~
~~Aulorhynchidae~~	~~*Aulorhynchus flavidus*~~	~~Tube-snout~~
~~Clupeidae~~	~~*Clupea pallasii*~~	~~Pacific herring~~
~~Embiotocidae~~	~~*Cymatogaster aggregate*~~	~~Shiner perch~~
~~Schophthalmidae~~	~~*Scophthalmus maxima*~~	~~Turbot~~

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following species, but an active infection has not been demonstrated: Common carp (*Cyprinus carpio*) and American yellow perch (*Perca flavescens*).

~~Family~~	~~Scientific name~~	~~Common name~~
~~Cyprinidae~~	~~*Cyprinus carpio*~~	~~Common carp~~
~~Percidae~~	~~*Perca flavescens*~~	~~American yellow perch~~

2.2.3. Non-susceptible species

None known.

2.2.4. Likelihood of infection by species, host life stage, population or sub-populations

IHNV predominantly infects ~~salmon and trout~~salmonid species with fry being the most highly susceptible stage (LaPatra, 1998). Resistance to infection typically increases with fish age until the spawning stage. Returning adult spawning salmon, can be highly infected and shed large amounts of virus in ovarian fluid and milt despite ~~a lack~~ the absence of clinical disease (Dixon *et al.,* 2016).

2.2.5. Distribution of the pathogen in the host

IHNV targets haematopoietic tissue and is most commonly isolated from kidney and spleen tissues. The virus has also been isolated from gill, oesophagus, intestine, stomach, pyloric caeca, liver, brain, heart, thymus, adipose tissue, muscle, skin, fin and mucous (Drolet *et al.,* 1994; Harmache *et al.,* 2006; LaPatra *et al.,* 1989; Yamamoto *et al.,* 1990). In spawning fish IHNV has also been isolated in ovarian fluid and milt (Mulcahy *et al*., 1982).

2.2.6. Aquatic animal reservoirs of infection

Field surveillance programmes and experimental infection trials have documented subclinical IHNV infections in various salmon and trout species (Knusel *et al.,* 2007; Mulcahy *et al.*, 1984; Pascoli *et al.,* 2015; St-Hilaire *et al.,* 2001; Traxler *et al.,* 1997). Survivors of laboratory exposures have demonstrated IHNV persistence for months to over one-year post-exposure (Drolet *et al.,* 1995; Foott *et al.,* 2006; Kim *et al.,* 1999; Muller *et al.,* 2015). With the exception of high viral load occurring in subclinically infected spawning adult salmon, the IHNV levels associated with subclinical infections tend to be lower than in fish undergoing clinical disease.

2.2.7. Vectors

A single study has demonstrated that adult salmon lice, *Lepeophtheirus salmonis* are capable of acquiring and transmitting IHNV to naïve Atlantic salmon through parasitism (Jakob *et al.,* 2011). Regardless of whether salmon lice acquired IHNV through water bath exposure or after parasitising IHNV-infected fish, the duration of virus association with salmon lice diminished rapidly with infectious virus levels falling below cell culture detection limits within hours. IHNV has also been isolated from freshwater invertebrates (e.g. leeches, copepods, and mayflies), however, their capacity to transmit virus is unknown (Dixon *et al.,* 2016; Garver & Wade, 2017).

2.3. Disease pattern

2.3.1. Mortality, morbidity and prevalence

Depending on the species of fish, rearing conditions, temperature, and virus strain, outbreaks of infection with IHNV may range from acute to chronic. An outbreak of infection with IHNV in farmed Atlantic salmon in British Colombia resulted in cumulative losses on affected farms between of 20 and 94% (Saksida, 2006). In chronic cases, losses are protracted and fish in various stages of disease can be observed in the pond. The prevalence of infection in chronic cases remains unknown. The limited available data indicated that prevalence of infection with IHNV can be high (59%) in endemically infected rainbow trout farms in Europe (reviewed by Dixon *et al*., 2016).

IHNV is endemic among populations of free-ranging salmonids throughout much of its historical range along the west coast of North America. Sockeye salmon have incurred losses of up to ~~99%~~ 36.9% at the fry stage (~~Kurath *et al.*, 2003~~ Meyers *et al.*, 2003). ~~As the fish~~With age~~s~~, the prevalence of infection decreases ~~with~~ in marine phase sockeye salmon smolts, and ~~the prevalence of infection~~ in adults is generally low (<15%) to undetectable. However, the prevalence of infection can again reach high levels in mature adult spawning sockeye salmon, with long-term studies revealing greater than 50% prevalence in wild populations (Meyers *et al.,* 2003).

2.3.2. Clinical signs, including behavioural changes

Fish with acute infection with IHNV can exhibit lethargy interspersed with bouts of frenzied, abnormal activity. During outbreaks, fish can display spiral swimming, flashing, and have trailing faecal casts. Fish may also show darkening of the skin, exophthalmia, distended abdomen and external haemorrhaging. In instances where fish survive an outbreak, spinal deformities may become evident (Bootland & Leong, 1999).

2.3.3 Gross pathology

Gross observations are non-pathognomonic and ~~can involve~~ may include ascites, pale gills, liver, kidney and spleen, petechial haemorrhaging, yellow mucous in the intestine and a lack of food in the stomach (Bootland & Leong, 1999; Traxler, 1986).

2.3.4. Modes of transmission and life cycle

The transmission of IHNV between fish is primarily horizontal through direct contact with virus contaminated water or via cohabitation with IHNV infected fish (Bootland & Leong, 1999). ~~However, cases of vertical or egg-associated transmission have been recorded (Mulcahy & Pascho, 1985).~~ There is insufficient evidence to demonstrate true vertical transmission. Outbreaks of IHNV as a result of egg movements likely occurred as a result of inadequate disinfection of moderaterly infected or untested broodstock (Dixon *et al.*, 2016). ~~While egg-associated transmission is significantly reduced by the now common practice of surface disinfection of eggs with an iodophor solution, it is the only mechanism accounting for the appearance of infection with IHNV in new geographical locations among fry originating from eggs that were incubated and hatched in virus-free water (Dixon *et al.*, 2016; Winton, 1991).~~

2.3.5. Environmental and management factors

The most important environmental factor affecting the disease progression is water temperature. Experimental trials have demonstrated that IHNV can produce mortality in water temperatures from 3°C to 18°C; however clinical disease typically occurs below 15°C under natural conditions (LaPatra, 1998).

2.3.6. Geographical distribution

Cases of infection with IHNV have been reported from Europe, Asia-Pacific, Africa and the Americas. For recent information on distribution at the country level consult the WAHIS interface (https://www.oie.int/wahis_2/public/wahid.php/Wahidhome/Home/index/newlang/en).

2.4. Biosecurity and disease control strategies

2.4.1. Vaccination

Plasmid DNA vaccines containing the gene for the IHNV glycoprotein have proven highly efficacious against infection with IHNV resulting in the licensing of one for commercial use in Atlantic salmon net-pen aquaculture on the west coast of North America (Alonso & Leong, 2013; Salonius *et al.,* 2007). Administered via intramuscular injection, an IHNV DNA vaccine was rapidly disseminated systemically followed by plasmid persistence in muscle at the injection site (Garver *et al.*, 2005); consequently, caution should be employed when testing fish vaccinated with the IHNV DNA vaccine as diagnostic methods targeting viral G-gene nucleotide sequence or protein have the potential to cross react with the vaccine.

2.4.2. Chemotherapy including blocking agents

Chemotherapeutics, including natural compounds, have been identified to have anti-IHNV properties; however, these have not found commercial use in aquaculture against IHNV (Winton, 1991). Direct application of anti-IHNV compounds to cell cultures has caused growth inhibition and toxicity that could affect the sensitivity of detecting IHNV in affected cultures (Balmer *et al.,* 2017; Hasobe & Saneyoshi, 1985).

2.4.3. Immunostimulation

Immunostimulants are not used commercially in aquaculture for IHNV (Ooi *et al.,* 2008).

2.4.4. Breeding resistant strains

Experimental trials of triploid or inter-species hybrids have been conducted (Barroso *et al.,* 2008; Winton, 1991) with resistance typically determined early in the infection process and associated with lower early viral replication (Purcell *et al*., 2010). However, no resistant strains are commercially available.

2.4.5. Inactivation methods

IHNV is readily inactivated by common disinfectants with active ingredients such as sodium hypochlorite, iodophor, benzalkonium chloride, saponated cresol, formaldehyde and potassium permanganate solution (Yoshimizu *et al.,* 2005). As these substances have virucidal properties any carry-over on sampling equipment or contact with samples may result in reduced viral titres.

2.4.6. Disinfection of eggs and larvae

Iodophor disinfection of eggs is a common practice to effectively mitigate egg-associated transmission of IHNV (Bovo *et al.,* 2005). Chapter 4.4. of the *Aquatic Code* provides recommendations for surface disinfection of salmonid eggs. Iodine has been shown to inhibit PCRs (Auinger *et al.,* 2008) and could affect PCR testing results of disinfected eggs.

2.4.7. General husbandry

In addition to disinfection of eggs (according to Chapter 4.4 of the *Aquatic Code*), use of a virus-free water supply and decreasing rearing densities have significant positive effects in the management of IHNV. Transmission of IHNV increases with host density (Ogut & Reno, 2004).

3. Specimen selection, sample collection, transportation and handling

This section draws on information in Sections 2.2, 2.3 and 2.4 to identify populations, individuals and samples which are most likely to be infected.

3.1. Selection of populations and individual specimens

Clinical inspections ~~are best~~ should be carried out during a period when~~ever~~ the water temperature is below 14°C, or whenever the water temperature is likely to reach its lowest annual point. All production units (ponds, tanks, net-cages, etc.) ~~must~~ should be inspected for the presence of dead, weak or abnormally behaving fish~~ of any susceptible species, and if they are present, such fish should be selected~~. Particular attention should be paid to the water outlet area, where weak fish tend to accumulate due to the water current.

For the purposes of disease surveillance, fish to be sampled are selected as follows: ~~If additional fish are required for the sample, healthy individuals should be selected as follows:~~

i) Rainbow trout and the other susceptible species listed in Section 2.2.1 should be sampled proportionally, or following risk-based criteria for targeted selection of lots or populations with a history of abnormal mortality or potential exposure events (e.g. via untreated surface water, wild harvest or replacement with stocks of unknown disease status). In farms with salmonids, if rainbow trout are present, only fish of that species should be selected for sampling. If rainbow trout are not present, the sample has to be obtained from fish of all other IHNV-susceptible species.

~~ii) Susceptible species should be sampled following risk-based criteria for targeted selection of populations with a history of abnormal mortality or potential exposure events (e.g. via untreated surface water, wild harvest or replacement with stocks of unknown risk status).~~

ii) If more than one water source is used for fish production, fish from all water sources should be included in the sample.

iii) If weak, abnormally behaving or freshly dead (not decomposed) fish are present, such fish should be selected. If such fish are not present, the fish selected should include normal appearing, healthy fish collected in such a way that all parts of the farm as well as all year classes are proportionally represented in the sample.

For disease outbreak investigations, moribund fish or fish exhibiting clinical signs of infection with IHNV should be collected. Ideally fish should be collected while alive, however recently dead fish can also be selected for diagnostic testing. It should be noted however, that there will be a significant risk of contamination with environmental bacteria if the animals have been dead for some time. There may be no clinical signs or gross pathognomonic lesions in cases of sudden mortality.

3.2. Selection of organs or tissues

The optimal tissues ~~material to be examined is~~ are spleen, anterior kidney, and either heart or brain. In the case of spawning fish, ovarian fluid and milt may be taken ~~examined~~.

In the case of small fry, whole fish less than 4 cm long can be homogenised (using, for example, sterile scissors or a scapel) after removal of the body behind the anal pore ~~gut opening~~. If a sample consists of whole fish with a body length between 4 cm and 6 cm, the viscera including kidney should be collected. For larger size fish, kidney, spleen, heart, encephalon, and ovarian fluid from brood fish at the time of spawning, should be the tissues to be sampled. ~~When possible,~~ Samples should be taken in duplicate to permit retesting if needed.

3.3. Samples or tissues not suitable for pathogen detection

IHNV is very sensitive to enzymic degradation, therefore sampling tissues with high enzymatic activities or large numbers of contaminating bacteria, such as the intestine or skin, should be avoided when possible. Given the haematopoietic nature of IHNV, muscle tissue should be avoided as a target tissue. The yolk sac of fry has also shown toxicity to cell lines and should be removed before inoculating cells for virus isolation. Preservatives and fixatives, such as RNAlater and formaldehyde can be toxic to tissue culture cells such as epithelioma papulosum cyprini (EPC) and fathead minnow (FHM), and can impact molecular detection methods (Auinger *et al.,* 2008; Pham *et al.,* 2018).

3.4. Non-lethal sampling

Ovarian fluid and milt are suitable samples for detection of IHNV in spawning adult salmon and trout (Dixon *et al.,* 2016; Meyers *et al.,* 2003). There is evidence that IHNV may be isolated from gill, fin and mucous samples but detection may be impacted by the state of infection, time since exposure and sample size (Burbank *et al.,* 2017; LaPatra *et al.,* 1989).

3.5. Preservation of samples for submission

For guidance on sample preservation methods for the intended test methods, see Chapter 2.3.0.

3.5.1. Samples for pathogen isolation

The success of pathogen isolation and results of bioassay depend strongly on the quality of samples (time since collection and time in storage). Fresh specimens should be kept on ice and preferably sent to the laboratory within 24 hours of collection. Alternate storage methods should only be used after consultation with the receiving laboratory.

Before shipment or transfer to the laboratory, pieces of the organs to be examined should be removed from the fish with sterile dissection tools and transferred to sterile plastic tubes containing transport medium, i.e. cell culture medium with 10% fetal calf serum (FCS) and antibiotics. The combination of 200 International Units (IU) penicillin, 200 µg streptomycin, and 200 µg kanamycin per ml are recommended, although other antibiotics of proven efficacy may also be used. The tissue in each sample should be larger than the analytical unit size required for initial laboratory testing (e.g. between 0.5 and 2 g) and taken in duplicate if retesting may be required.

Tubes containing fish tissues in transport medium for cell cultivation should be placed in insulated containers, such as thick-walled polystyrene boxes, together with sufficient ice or an alternative cooling medium with the similar cooling effect to ensure chilling of the samples during transportation to the laboratory. However, freezing of the samples should be avoided. The temperature of a sample during transit must never exceed 10°C, and ice must still be present in the transport box at receipt or at least one ~~or more~~ freeze block~~s~~ must still be partly or completely frozen.

Whole fish may be sent to the laboratory if the temperature requirements referred to in the first paragraph during transportation can be fulfilled. Whole fish should be wrapped up in paper with absorptive capacity and enclosed in a plastic bag. Live fish may also be transported to the laboratory. All packaging and labelling must be performed in accordance with present national and international transport regulations, as appropriate.

The virological examination on cell culture should be started as soon as possible, and no later than 48 hours after the collection of the samples. In exceptional cases, the virological examination may be started at the latest within 72 hours after the collection of the material, provided that the material to be examined is protected by a transport medium, and that the temperature requirements during transportation can be fulfilled.

3.5.2. Preservation of samples for molecular detection

Samples can be taken from the fish in accordance with the procedure described in Section 3.5.1., using a sterile instrument, and transferred to a sterile plastic tube containing transport medium.

Alternatively, samples may be placed in at least five volumes of RNA stabilisation reagents, according to the recommendation from the manufacturers. Samples in RNA stabilising reagents can be shipped on ice or at room temperature if transport time does not exceed 24 hours.

Whole fish may also be sent to the laboratory (see Section 3.5.1).

3.5.3. Fixed samples for histopathology, immunohistochemistry or *in-situ* hybridisation

Tissue samples for histopathology should be immediately fixed at a fixative to tissue ratio of 10:1. A suitable fixative is 10% buffered formalin. To avoid excessive cross-linking, tissue should be transferred to ethanol after 24hrs if methods other than histopathology are used e.g. *in-situ* hybridisation.

3.5.4. Fixed samples for electron microscopy

~~Not relevant.~~ Samples for electron microscopy are not routinely required and are collected only when it is considered beneficial to facilitate further diagnostic investigation. A 2 mm cubed section from each of the appropriate organs described in section 3.2 should be fixed in glutaraldehyde; the recommended ratio of fixative to tissue is 10:1.

3.5.5. Samples for other tests

Not relevant.

3.6. Pooling of samples

~~No data are currently available concerning the effect of pooling samples on the detection of IHNV. However, small life stages such as fry can be pooled to provide the minimum amount of material needed for testing.~~ Pooling of samples from more than one individual animal for a given purpose should only be recommended where supporting data on diagnostic sensitivity and diagnostic specificity are available. However, smaller life stages (e.g. fry) can be pooled to provide a minimum amount of material for testing.

4. Diagnostic methods

The methods currently available for identifying infection that can be used in i) surveillance of apparently healthy populations), ii) presumptive and iii) confirmatory diagnostic purposes are listed in Table 4.1. by life stage. The designations used in the Table indicate:

Key:

+++ =	Recommended method(s) validated for the purpose shown and usually to stage 3 of the OIE Validation Pathway;
++ =	Suitable method(s) but may need further validation;
+ =	May be used in some situations, but cost, reliability, lack of validation or other factors severely limits its application;
Shaded boxes =	Not appropriate for this purpose.

The selection of a test for a given purpose depends on the analytical and diagnostic sensitivities and specificities repeatability and reproducibility. OIE Reference Laboratories welcome feedback on diagnostic performance for assays, in particular PCR methods, for factors affecting assay analytical sensitivity or analytical specificity, such as tissue components inhibiting amplification, presence of nonspecific or uncertain bands, etc., and any assays that are in the +++ category.

Table 4.1. *OIE recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals*

Method	A. Surveillance of apparently healthy animals				B. Presumptive diagnosis of clinically affected animals				C. 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
Wet mounts												
Histopathology[3]						++	++	1				
Cytopathology[3]												
Cell or artificial media culture	+++	+++	+++	3	+++	+++	+++	3	+++	+++	+++	3
Real-time PCR	+++	+++	+++	3	+++	+++	+++	3	+++	+++	+++	3
Conventional PCR						++	++	~~++~~ 2	++	++	++	1
Amplicon sequencing[4]									+++	+++	+++	3
In-situ hybridisation												
Bioassay												
LAMP												
IFAT									++	++	++	2
Ag-ELISA									++	++	++	2
Neutralisation test (antibody or antiserum)[5]									++	++	++	2

LV = level of validation, refers to the stage of validation in the OIE Pathway (Chapter 1.1.2); PCR = polymerase chain reaction; LAMP = loop-mediated isothermal amplification. IFAT = indirect fluorescent antibody test; Ag-ELISA = antigen enzyme-linked immunosorbent assay. [1]For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). [2]Early and juvenile life stages have been defined in Section 2.2.4.

[3]Cytopathology and histopathology can be validated if the results from different operators has been statistically compared.

[4]Sequencing of the PCR product.

[5]Specify the test used. Shading indicates the test is inappropriate or should not be used for this purpose.

4.1. Wet mounts

Not relevant

4.2. Histopathology and cytopathology

Histopathological findings reveal degenerative necrosis in haematopoietic tissues, kidney, spleen, liver, pancreas, and digestive tract. Necrosis of eosinophilic granular cells in the intestinal wall is pathognomonic of IHNV infection (Bootland & Leong, 1999).

The blood of affected fry shows reduced haematocrit, leukopenia, degeneration of leucocytes and thrombocytes, and large amounts of cellular debris. As with other haemorrhagic viraemias of fish, blood chemistry is altered in severe cases (Bootland & Leong, 1999).

Electron microscopy of virus-infected cells reveals bullet-shaped virions of approximately 150–190 nm in length and 65–75 nm in width (Wolf, 1988). ~~The~~ Virions are visible at the cell surface or within vacuoles or intracellular spaces after budding through cellular membranes. The virion possesses an outer envelope containing host lipids and the viral glycoprotein spikes that react with immunogold staining to decorate the virion surface

Smears are not appropriate for detection or identification of IHNV.

4.3. Cell or artificial media culture for isolation

4.3.1. Cell lines

The recommended cell lines for IHNV detection are EPC or FHM. Cell lines should be monitored to ensure that susceptibility to targeted pathogens has not changed.

EPC or FHM cells are grown at 20–30°C in suitable medium, e.g. Eagle's minimal essential medium (MEM; or modifications thereof) with a supplement of 10% fetal bovine serum (FBS) and antibiotics in standard concentrations. When the cells are cultivated in closed vials, it is recommended to buffer the medium with bicarbonate. The medium used for cultivation of cells in open units may be buffered with Tris-HCl (23 mM) and Na-bicarbonate (6 mM). The pH must be 7.6 ± 0.2. Cell culture plates should be seeded 4–48 hours and not 100% confluent prior to inoculation. 15–30 minutes prior to sample inoculation, cells should be pre-treated with 7% (w/v) PEG-20,000 solution (10–15 µl/cm^2) (Batts & Winton, 1989).

4.3.2. Sample preparation and inoculation

Note: Tissue and fluid samples should be kept cool throughout sample preparation procedures.

i) Homogenise tissue samples using mortar and pestle or a tissue homogeniser~~, stomacher, polytron or equivalent~~. A small volume of media (MEM-4 or Hank's balanced salt solution with antibiotics) may be needed to achieve complete homogenisation.

ii) Adjust the volume of media to a final ratio of 10:1 (media:tissue) and mix thoroughly. For fluid samples adjust the volume of media to a final ratio of 1:1.

iii) Centrifuge the homogenate or fluid samples at 2000–4000 ***g*** for 15 minutes at 2–5°C.

iv) Remove the supernatant and pass through a 0.45 µM membrane filter (if available).

v) If the sample cannot be inoculated within 48 hours after collection, the supernatant may be stored at –80°C provided virological examination is carried out within 14 days.

vi) If samples originate from an area where infectious pancreatic necrosis virus (IPNV) is present, supernatants may be treated with IPNV antiserum. Mix the supernatant with equal parts of a suitably diluted pool of antisera to the indigenous serotypes of IPNV and incubate for a minimum of 1 hour at 15°C or up to 18 hours at 4°C. The titre of the antiserum must be at least 1/2000 in a 50% plaque neutralisation test.

vii) Samples are inoculated into cell cultures in at least two dilutions, i.e. the primary dilution and a 1:10 dilution thereof, resulting in final dilutions of tissue material in cell culture medium of 1:100 and 1:1000, respectively. The ratio between inoculum size and volume of cell culture medium should be about 1:10. For each dilution and each cell line, a minimum of about 2 cm^2 cell area, corresponding to one well in a 24-well cell culture tray, has to be used. Use of cell culture trays is recommended, but other units of similar or with larger growth area are acceptable as well.

viii) Inoculated cell cultures are incubated at 15°C for 7–10 days. Using a microscope with 40–150× magnification, cultures should be inspected for toxicity the day after inoculation, particularly if supernatant was not filtered in step iv. The use of a phase-contrast microscope is recommended.

ix) ~~Monitor t~~The cells are monitored regularly (2–3 times a week) for the presence of cytopathic effect (CPE).

Interpretation of results

If CPE is observed, confirmatory testing is required to identify IHNV.

If no CPE is observed in the primary culture or subcultivation, the sample is negative.

4.4. Nucleic acid amplification

4.4.1. Real-time PCR

There are several ~~reverse-transcription~~ real-time reverse-transcription (RT) PCR assays available for the detection of IHNV. Two assays are described, a two-step real-time PCR and a one-step real-time PCR.

The first assay described is a stage 3 validated two-step real-time TaqMan PCR assay that amplifies a region of the nucleoprotein gene of all known IHNV genogroups with some E-genogroup isolates (D332-92, FV23, and FV91-40) having reduced amplification efficiency due to single nucleotide polymorphism within the probe sequence (Hoferer *et al.,* 2019; Purcell *et al.,* 2013).

Positive and negative controls should be run with each stage of the assay: extraction, reverse transcription and real-time PCR. Due to the sensitive nature of PCR-based assays, it is important to be able to distinguish a true positive from the positive control material. This may be achieved using an artificial positive control as employed by Purcell *et al.* (2013). It is also highly recommended that master mix, template addition and PCR amplification occur in designated hoods or spatially separated areas.

RNA extraction and reverse-transcription (*RT*)

i) Total RNA from infected cells and/or tissues is extracted using a phase-separation method (e.g. phenol-chloroform or Trizol) or by use of a commercially available RNA isolation kit used according to the manufacturer's instructions.

ii) Extracted RNA is reverse transcribed non-discriminately into cDNA using random primers. The cDNA synthesis reactions and cycling conditions are best performed using the manufacturer's instructions for commercially available kits which have been extensively tested with a variety of RNA templates, including GC- and AU-rich targets and RNAse expressed at low levels.

Real-time PCR

The TaqMan real-time PCR assay uses forward primer IHNv N 796F (5'-AGA-GCC-AAG-GCA-CTG-TGC-G-3'), reverse primer IHNv N 875R (5'-TTC-TTT-GCG-GCT-TG-GTT-GA-3') and FAM-labelled probe, IHNv N 818T (5'-6FAM-TGA-GAC-TGA-GCG-GGA-CA-MGBNFQ-3'). Primers are used at a final concentration of 900 nM each and the final probe concentration is 250 nM. 2.5 µl cDNA product is added to each 25 µl rPCR reaction. Thermal cycling conditions are 50°C 2 minutes, 95°C 10 minutes followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute.

The sample is negative if no Ct (threshold cycle) is recorded, while samples with a Ct are considered positive for IHNV.

One step real-time RT-PCR

The one step real-time RT-PCR is performed using the SuperScript III Platinum One-Step qRT-PCR Kit (Thermo Fisher Scientific, Schwerte, Germany) and/or the AgPath-ID One-Step RT-PCR Kit (Thermo Fisher Scientific) according to the manufacturers' instructions. For all quantitative assays, the following unique parameters were used: (a) total volume of 25 µl consisting of 20 µl mastermix and 5 µl of RNA; (b) 900 nM of each primer; (c) 200 nM of IHNV probe and 250 nM of VHSV probe, respectively; (d) hard-shell 96-well skirted plates with white shell (Bio-Rad, Munich, Germany, cat. No HSP9601); (e) Microseal B adhesive optical clear seals (Bio-Rad, cat. no MSB 1001); (f) run on a C1000TM Thermal Cycler controlled by the CFX96TM Real-Time PCR Detection System (Bio-Rad); and (g) use of the CFX Manager software (Bio-Rad) for data analysis. The threshold was set automatically (Hoferer *et al.,* 2019)

4.4.2. Conventional PCR

Several conventional PCR assays are available with limited validation data.

The PCR assay described recognises a broad range of genotypes by targeting a central region of the IHNV G gene (Emmenegger *et al.,* 2000), and produces a PCR amplicon that is used for identification of genetic strains and for epidemiological tracing of virus movements (Kurath *et al.,* 2003).

Positive and negative controls should be run with each stage of the assay: extraction, RT-PCR and second round PCR. Due to the sensitive nature of PCR-based assays it is highly recommended that master mix, template addition and PCR amplification occur in designated hoods or spatially separated areas.

RNA extraction

Total RNA may be prepared as described in section 4.4.1.

Conventional RT-PCR (Round 1)

The first round RT-PCR combines cDNA synthesis and PCR amplification into one step by using an IHNV-specific primer set that generates the first-strand synthesis of IHNV RNA and subsequent PCR amplification through 30 cycles. The first round PCR produces a 693 bp PCR amplicon using forward primer (5'-AGA-GAT-CCC-TAC-ACC-AGA-GAC-3') and reverse primer (5'-GGT-GGT-GTT-GTT-TCC-GTG-CAA-3') at a final concentration of 200 nM each. The thermal cycling conditions are one cycle of 50°C for 30 minutes; one cycle of 95°C for 2 minutes; 30 cycles of 95°C for 30 seconds, 50°C for 30 seconds, 72°C for 60 seconds; one cycle of 72°C for 7 minutes and 4°C hold.

A sample is IHNV positive if a 693 bp PCR amplicon is observed and no bands were observed in the negative controls. If no band is observed for a sample and the positive controls passed proceed to the second round nested PCR.

Second round (nested PCR)

Due to the sensitivity of the test along with the need for repetitive handling of tubes, nested PCR is prone to contamination and good sterile technique must be practiced.

The first round positive and negative controls are carried over and included with the nested PCR assay. In addition, a separate negative and positive control specific to the nested assay are required.

The second round PCR produces a 483 bp PCR amplicon using forward primer (5'-TCA-CCC-TGC-CAG-ACT-CAT-TGG-3') and reverse primer (5'-ATA-GAT-GGA-GCC-TTT-GTG-CAT-3') at a final concentration of 200 nM each. The thermal cycling conditions are: 95°C for 2 minutes followed by 30 cycles of 95°C for 30 seconds, 50°C for 30 seconds, 72°C for 60 seconds; one cycle of 72°C for 7 minutes and 4°C hold.

A sample is IHNV positive if a 483 bp PCR amplicon is observed and no band(s) are observed in the negative controls. A sample is negative if no bands are observed and positive controls passed.

4.4.3. Other nucleic acid amplification methods

To date, no other nucleic acid amplification method capable of universal IHNV detection has been sufficiently validated.

4.5. Amplicon sequencing

Nucleotide sequencing of the conventional PCR product (Section 4.4.2) is recommended as one of the final steps for confirmatory diagnosis. This central region of IHNV glycoprotein gene is used for identification of genetic strains and for epidemiological study (Kurath *et al.,* 2003). It is recommended to forward any sequence data obtained to the OIE Reference Laboratory, particularly in the event where isolate sequences differ from any of the target sequences of the recommended molecular assays.

4.6. *In-situ* hybridisation

Not relevant.

4.7. Immunohistochemistry

Not relevant.

4.8. Bioassay

Not relevant.

4.9. Antibody- or antigen-based detection methods

Antibody- and antigen-based detection methods may be used to confirm the presence of IHNV in cell culture. Kits and antibodies are commercially available and should be used according to manufacturer's instructions. Sensitivity, specificity and sample preparation can influence the results; a negative result should be viewed with caution. These techniques should not be used as a screening method.

4.9.1. Neutralisation test (identification in cell culture)

i) Collect the culture medium of the cell monolayers exhibiting CPE and centrifuge an aliquot at 2000 g for 15 minutes at 4°C, or filter through a 0.45 µm (or 450 nm) pore membrane to remove cell debris.

ii) Dilute virus-containing medium from 10^2–10^4.

iii) Mix aliquots (for example 200 µl) of each dilution with equal volumes of an IHNV antibody solution. The neutralising antibody solution must have a 50% plaque reduction titre of at least 2000. Likewise, treat a set of aliquots of each virus dilution with cell culture medium to provide a non-neutralised control.

iv) In parallel, a neutralisation test must be performed against a homologous IHNV strain (positive neutralisation test) to confirm the reactivity of the antiserum.

v) Incubate all the mixtures at 15°C for 1 hour.

vi) Transfer aliquots of each of the above mixtures on to 24-hour-old monolayers overlaid with cell culture medium containing 10% FBS (inoculate two wells per dilution) and incubate at 15°C; 24- or 12-well cell culture plates are suitable for this purpose, using a 50 µl inoculum.

vii) Check the cell cultures for the onset of CPE and read the results for each suspect IHNV sample and compare to the occurrence of CPE of non-neutralised controls. Results are recorded either after a simple microscopic examination (phase contrast preferable) or after discarding the cell culture medium and staining cell monolayers with a solution of 1% crystal violet in 20% ethanol.

viii) The tested virus is identified as IHNV when CPE is prevented or noticeably delayed in the cell cultures that received the virus suspension treated with the IHNV-specific antibody, whereas CPE is evident in all other cell cultures.

Other neutralisation tests of demonstrated performance may be used instead.

4.9.2. Indirect fluorescent antibody test (IFAT) (identification in cell culture)

i) Prepare monolayers of cells in 2 cm^2 wells of cell culture plastic plates or on cover slips in order to reach around 80% confluency, which is usually achieved within 24 hours of incubation at ~~22°C~~ the optimal temperature of the cell line in question (e.g. 26°C for EPC and 20°C for RTG) (seed six cell monolayers per virus isolate to be identified, plus two for positive and two for negative controls). The FBS content of the cell culture medium can be reduced to 2–4%. If numerous virus isolates have to be identified, the use of black 96-well plates for immunofluorescence is recommended.

ii) When the cell monolayers are ready for infection (i.e. on the same day or on the day after seeding) inoculate the virus suspensions to be identified by making tenfold dilution steps directly in the cell culture wells or flasks.

iii) Dilute the control virus suspension of IHNV in a similar way, in order to obtain a virus titre of about 5,000–10,000 plaque-forming units (PFU)/ml in the cell culture medium.

iv) Incubate at 15°C for 24 hours.

v) Remove the cell culture medium, rinse once with 0.01 M phosphate buffered saline (PBS), pH 7.2, then three times briefly with a cold mixture of acetone 30%/ethanol 70% (v/v) (stored at –20°C).

vi) Let the fixative act for 15 minutes. A volume of 0.5 ml is adequate for 2 cm^2 of cell monolayer.

vii) Allow the cell monolayers to air-dry for at least 30 minutes and process immediately or freeze at –20°C.

viii) Prepare a solution of purified IHNV antibody or serum in 0.01 M PBS, pH 7.2, containing 0.05% Tween-80 (PBST), at the appropriate dilution (which has been established previously or is given by the reagent supplier).

ix) Rehydrate the dried cell monolayers by four rinsing steps with the PBST solution and remove this buffer completely after the last rinsing.

x) Treat the cell monolayers with the antibody solution for 1 hour at 37°C in a humid chamber and do not allow evaporation to occur (e.g. by adding a piece of wet cotton to the humid chamber). The volume of solution to be used is 0.25 ml/2 cm^2 well.

xi) Rinse four times with PBST as above.

xii) Treat the cell monolayers for 1 hour at 37°C with a solution of fluorescein isothiocyanate- or tetramethylrhodamine-5-(and-6-) isothiocyanate-conjugated antibody to the immunoglobulin used in the first layer and prepared according to the instructions of the supplier. These conjugated antibodies are most often rabbit or goat antibodies.

xiii) Rinse four times with PBST.

xiv) Examine the treated cell monolayers on plastic plates immediately, or mount the cover slips using, for example, glycerol saline, pH 8.5 prior to microscopic observation.

xv) Examine under incident UV light using a microscope with × 10 eye pieces and × 20–40 objective lens having numerical aperture >0.65 and >1.3, respectively. Positive and negative controls must be found to give the expected results prior to any other observation.

Other IFAT or immunocytochemical (alkaline phosphatase or peroxidase) techniques of demonstrated performance may be used instead.

4.9.3. Enzyme-linked immunosorbent assay (ELISA)

i) Coat the wells of microplates designed for ELISAs with appropriate dilutions of purified immunoglobulins (Ig) or serum specific for IHNV, in 0.01 M PBS, pH 7.2 (200 µl/well).

ii) Incubate overnight at 4°C.

iii) Rinse four times with 0.01 M PBS containing 0.05% Tween-20 (PBST).

iv) Block with skim milk (5% in PBST) or other blocking solution for 1 hour at 37°C (200 µl/well).

v) Rinse four times with PBST.

vi) Add 2% Triton X-100 to the virus suspension to be identified.

vii) Dispense 100 µl/well of two- or four-step dilutions of the virus to be identified and of IHNV control virus, and a heterologous virus control (e.g. viral haemorrhagic septicaemia virus). Allow the samples to react with the coated antibody to IHNV for 1 hour at 20°C.

viii) Rinse four times with PBST.

ix) Add to the wells either biotinylated polyclonal IHNV antiserum or MAb to N protein specific for a domain different from the one of the coating MAb and previously conjugated with biotin.

x) Incubate for 1 hour at 37°C.

xi) Rinse four times with PBST.

xii) Add streptavidin-conjugated horseradish peroxidase to those wells that have received the biotin-conjugated antibody, and incubate for 1 hour at 20°C.

xiii) Rinse four times with PBST. Add the substrate and chromogen. Stop the course of the test when positive controls react and read the results.

xiv) Interpretation of the results is according to the optical absorbencies achieved by negative and positive controls and must follow the guidelines for each test, e.g. absorbency at 450 nm of positive control must be minimum 5–10 × A450 of negative control.

The above biotin–avidin-based ELISA version is given as an example. Other ELISA versions of demonstrated performance may be used instead.

4.10. Other ~~serological~~ methods

Not applicable

5. Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations

Virus isolation in cell culture or real-time RT-PCR are the recommended tests for surveillance to demonstrate freedom from infection with IHNV.

6. Corroborative diagnostic criteria

This section only addresses the diagnostic test results for detection of infection in the absence (Section 6.1.) or in the presence of clinical signs (Section 6.2.) but does not evaluate whether the infectious agent is the cause of the clinical event.

The case definitions for a suspect and confirmed case have been developed to support decision making related to trade and confirmation of disease status at the country, zone or compartment level. Case definitions for disease confirmation in endemically affected areas may be less stringent. It is recommended that all samples that yield suspect positive test results in an otherwise pathogen-free country or zone or compartment should be referred immediately to the OIE Reference Laboratory for confirmation, whether or not clinical signs are associated with the case. If a laboratory does not have the capacity to undertake the necessary diagnostic tests it should seek advice from the appropriate OIE Reference Laboratory.

6.1. Apparently healthy animals or animals of unknown health status[3]

Apparently healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link to an infected population. Geographic proximity to, or movement of animals or animal products or equipment, etc., from a known infected population equate to an epidemiological link. Alternatively, healthy populations are sampled in surveys to demonstrate disease freedom.

[3] For example transboundary *commodities*.

6.1.1. Definition of suspect case in apparently healthy animals

The presence of infection with IHNV shall be suspected if at least one of the following criteria is met:

i) Positive result by real-time RT-PCR;

ii) IHNV-typical CPE ~~Cytopathic effect~~ in cell culture.

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with IHNV is considered to ~~shall~~ be confirmed if, in addition to the criteria in Section 6.1.1., ~~positive results has been obtained on at least one animal from two test used in the following combination~~ one or more of the following criteria is met:

i) Positive result by real-time ~~RT~~ RT-PCR ~~followed by~~and detection of IHNV in a tissue sample by ~~a positive result from a~~ conventional PCR targeting a non-overlapping region of the genome and amplicon sequencing;

ii) CPE ~~Isolation of virus~~ in cell culture ~~confirmed by~~ identified as IHNV by real-time RT-PCR, conventional PCR, IFAT, ~~or~~ Ag-ELISA, or by a neutralisation test ~~and a positive result followed by~~and detection of IHNV in a tissue sample by real-time RT-PCR;

iii) CPE ~~Isolation of virus~~ in cell culture ~~confirmed by~~ identified as IHNV by real-time RT-PCR, conventional PCR, IFAT, ~~or~~ Ag-ELISA, or by a neutralisation test ~~and followed by~~and detection of IHNV in a tissue sample by conventional PCR and amplicon sequencing;

~~iv) Positive result by real-time RT-PCR followed by isolation of virus in cell culture confirmed by identified as IHNV by real-time RT-PCR, conventional PCR, IFAT, Ag-ELISA, or by a neutralisation test and amplicon sequencing.~~

Reference Laboratories should be contacted for specimen referral when testing laboratories cannot undertake any of the recommended test methods and testing is being undertaken that will result in notification to the OIE.

6.2. Clinically affected animals

Clinical signs are not pathognomonic for a single disease; however, they may narrow the range of possible diagnoses.

6.2.1. Definition of suspect case in clinically affected animals

The presence of infection with IHNV shall be suspected if at least one of the following criteria is met:

i) Gross pathology or clinical signs associated with the disease as described in this chapter, with or without elevated mortality;

ii) Positive result by real-time RT-PCR;

iii) IHNV-typical CPE ~~Cytopathic effect~~ in cell culture.

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with IHNV is considered to ~~shall~~ be confirmed if, in addition to the criteria in Section 6.2.1., ~~positive results has been obtained on at least one animal from two tests used in the following combination~~ one or more of the following criteria is met:

i) Positive result by real-time ~~RT~~ RT-PCR ~~followed by~~and detection of IHNV in a tissue sample ~~a positive result from a~~ conventional PCR targeting a non-overlapping region of the genome and amplicon sequencing;

ii) CPE ~~Isolation of virus~~ in cell culture ~~confirmed by~~ identified as IHNV by real-time RT-PCR, conventional PCR, IFAT, ~~or~~ Ag-ELISA, or by a neutralisation test ~~and a positive result followed by~~and detection of IHNV in a tissue sample by real-time RT-PCR;

iii) CPE ~~Isolation of virus~~ in cell culture ~~confirmed by~~ identified as IHNV by real-time RT-PCR, conventional PCR, IFAT, ~~or~~ Ag-ELISA, or by a neutralisation test ~~and followed by~~and detection of IHNV in a tissue sample by conventional PCR and amplicon sequencing;

~~iv) Positive result by real-time RT-PCR followed by isolation of virus in cell culture confirmed by identified as IHNV by real-time RT-PCR, conventional PCR, IFAT, Ag-ELISA, or by a neutralisation test and amplicon sequencing~~.

Reference Laboratories should be contacted for specimen referral when testing laboratories cannot undertake any of the recommended test methods and testing is being undertaken that will result in notification to the OIE.

6.3. Diagnostic sensitivity and specificity for diagnostic tests

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with IHNV are provided in Table 6.3. This information can be used for the design of surveys for infection with IHNV, however, it should be noted that diagnostic performance is specific to the circumstances of each diagnostic accuracy study (including the test purpose, source population, tissue sample types and host species) and diagnostic performance may vary under different conditions. Data is only presented where tests are validated to atleast level two of the validation pathway described in Chapter 1.1.2. and the information is available within published diagnostic accuracy studies.

Table 6.1. *Diagnostic performance of tests recommended for surveillance or diagnosis*

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation
Real-time RT-PCR	Diagnosis	Experimentally infected salmon	Kidney	Steelhead (*Oncorhynchus mykiss*)	100 (50)	100 (50)	Animals of known infection status	Purcell *et al.*, 2013
RT-PCR (single step)	Diagnosis	Experimentally infected salmon	Kidney	Steelhead (*Oncorhynchus mykiss*)	58 (50)	100 (50)	Animals of known infection status	Purcell *et al.*, 2013
Virus Isolation	Diagnosis	Experimentally infected salmon	Kidney	Steelhead (*Oncorhynchus mykiss*)	84 (50)	100 (50)	Animals of known infection status	Purcell *et al.*, 2013
		Field samples	Kidney and spleen	Atlantic salmon (*Salmo salar*)	80-86 (50)	100 (50)	Clinical signs – history	McClure *et al.*, 2008

DSe = diagnostic sensitivity; DSp = diagnostic specificity; *n* = number of samples used in the study;
~~RT-LAMP: = real-time loop mediated isothermal amplification. *Listed as suitable test~~

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NB: There are OIE Reference Laboratories for Infection with infectious haematopoietic necrosis virus (see Table at the end of this *Aquatic Manual* or consult the OIE web site for the most up-to-date list: http://www.oie.int/en/scientific-expertise/reference-laboratories/list-of-laboratories/).
Please contact the OIE Reference Laboratories for any further information on infection with viral haemorrhagic septicaemia virus

NB: FIRST ADOPTED IN 1995 AS INFECTIOUS HAEMATOPOIETIC NECROSIS; MOST RECENT UPDATES ADOPTED IN 2019.

CHAPTER 2.3.10.

INFECTION WITH VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUS

1. Scope

Infection with viral haemorrhagic septicaemia virus (VHSV) means infection with the pathogenic agent viral haemorrhagic septicaemia virus of the Genus *Novirhabdovirus* and Family *Rhabdoviridae.*

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

VHSV is a bullet-shaped particle, approximately 70 nm in diameter and 180 nm in length, that contains a negative-sense, single-stranded RNA genome of approximately 11,000 nucleotides, and possesses an envelope that contains the membrane glycoprotein, which is the neutralising surface antigen. The genome encodes six proteins: a nucleoprotein N; a phosphoprotein P (formerly designated M1); a matrix protein M (formerly designated M2); a glycoprotein G; a non-virion protein NV and a polymerase L (Walker *et al*., 2000).

G-gene nucleotide sequences have been used to classify VHSV isolates into four major genotypes (I, II, III and IV) and nine subtypes (Ia–Ie and IVa-IVd) with almost distinct geographical distributions (Einer-Jensen *et al*., 2004; Elsayed *et al*., 2006). The host range and the pathogenicity appear, at least to some extent, to be linked to the genotype of VHSV.

i) Genotype Ia

Almost all VHSV isolates causing outbreaks in European rainbow trout (*Oncorhynchus mykiss*) farms cluster in sub-lineage Ia, of which isolates have been reported from most continental European countries (Einer-Jensen *et al*., 2004; Kahns *et al*., 2012; Snow *et al*., 2004; Toplak *et al*., 2010). However, genotype Ia isolates have also been detected in other species in Europe such as brown trout (*Salmo trutta*), pike (*Esox lucius*) and grayling (*Thymallus thymallus*) (de Kinkelin & Le Berre, 1977; Jonstrup *et al*., 2009). Genotype Ia isolates have generally caused outbreaks in European freshwater farms, but isolates have also been obtained from rainbow trout in seawater net pens and turbot (*Scophthalmus maximus*~~ syn. *Psetta maxima*~~) (Schlotfeldt *et al*., 1991; Snow *et al*., 2004). Genotype Ia can be further subdivided into two major subpopulations, Ia-1 and Ia-2, with a distinct geographic distribution within Europe (Kahns *et al*., 2012).

ii) Genotype Ib

The isolates included in this genotype~~ Viruses~~ have been ~~isolated~~ obtained from fish in the marine environment in the Baltic Sea, Kattegat, Skagerrak, the North Sea and the English Channel (Einer-Jensen *et al*., 2004; Skall *et al*., 2005b; Snow *et al*., 2004) and as far north as latitude 70°N close to Nordkapp in Norway (Sandlund *et al.,* 2014). A single case was observed in Japan (Nishizawa *et al*., 2002). None of the isolations from wild fish has been associated with clinical disease outbreaks (Johansen *et al.,* 2013). Genotype Ib has been associated with evidence of transfer between wild fish and farmed rainbow trout in only two cases in pen-reared rainbow trout in Sweden in 1998 and 2000 (Nordblom, 1998; Nordblom & Norell, 2000; Skall *et al*., 2005a).

iii) Genotype Ic

This genotype consists of ~~is~~ a smaller group ~~consisting~~ of Danish isolates from farmed rainbow trout ~~isolates from earlier dates~~. Isolates of this genotype have also been ~~identified~~ detected in Germany and Austria (Jonstrup *et al*., 2009).

iv) Genotype Id

~~This group~~ The isolates included in this genotype consists of some old Scandinavian isolates from the 1960s until the first ~~VHS~~ outbreaks of infection with VHSV occurred in Finland in sea-reared rainbow trout in 2000. These outbreaks occurred in ~~at~~ two different areas ~~where~~ and all of the isolates sampled were ~~proved to~~ clustered in the Id genotype group. In infection trials, it was demonstrated that the isolates were pathogenic to rainbow trout, but less virulent than most Ia isolates (Raja-Halli *et al*., 2006).

v) Genotype Ie

The~~se~~ isolates included in this genotype have been obtained from both freshwater and marine (the Black Sea) environments in Georgia and Turkey. Isolations were from both farmed and wild turbot (Jonstrup *et al*., 2009; Kalayci *et al*., 2006; Nishizawa *et al*., 2006) and from rainbow trout (Einer-Jensen *et al*., 2004). VHSV Ie has also been ~~identified~~ isolated from ~~in~~ whiting (*Merlangius merlangus*) and sea bass (*Dicentrarchus labrax*) ~~from~~ in the Black Sea (Altuntas & Ogut, 2010).

vi) Genotype II

The ~~members~~ isolates included in ~~of~~ this ~~group~~ genotype ~~consist of~~ have been primarily detected in marine ~~isolates from~~ wild fish, in particular ~~especially from~~ Atlantic herring (*Clupea harengus*), from ~~in~~ the Baltic Sea, including the Gulf of Bothnia and the Gulf of Finland, (Gadd *et al*., 2011; Snow *et al*., 2004). Genotype II isolates have also been detected in lamprey (*Lampetra fluviatilis*) caught in freshwater from the rivers Kalajoki and Lestijoki, which have~~ing~~ an outlet into the Gulf of Bothnia (Gadd *et al*., 2010).

vii) Genotype III

The~~se~~ isolates included in this genotype originate from wild and farmed fish in the North Atlantic Sea from the Flemish Cap (Lopez-Vazquez *et al*., 2006b) to the Norwegian coast (Dale *et al*., 2009), the North Sea, around the British Isles, Skagerrak and Kattegat. ~~VHS outbreaks~~ Outbreaks of infection with VHSV in farmed turbot in the United Kingdom and Ireland in the 1990s were attributed ~~due~~ to infection with genotype III isolates, and in 2007 an outbreak in sea-reared rainbow trout at the Norwegian west coast was due to VHSV genotype III. ~~VHS~~ Outbreaks of infection with VHSV in five species of wrasse used as cleaner fish around the Shetland Islands were also due to this genotype (Munro *et al*., 2015).

viii) Genotype IVa

The isolates included in this genotype have been detected ~~originate~~ in finfish from both the east and west coasts of North America, as well as from the Asian countries of South Korea and Japan. Genotype IVa isolates in North America have caused severe epidemics in numerous wild marine species such as Pacific herring (*Clupea pallasii pallasii*) (Meyers & Winton, 1995), which can serve as a reservoir of virus to sympatric net-pen farmed Atlantic salmon (*Salmo salar*) (Garver *et al*., 2013). In Asia, genotype IVa isolates have caused disease outbreaks in ~~olive flounder~~ bastard halibut (*Paralichthys olivaceus*) (Ogut & Altuntas, 2014).

ix) Genotype IVb

The isolates included in this genotype have been detected ~~originate~~ in finfish ~~originate~~ from the North America Laurentian Great Lakes region (Gagne *et al*., 2007; Thompson *et al*., 2011; Winton *et al*., 2008) and ~~where they~~ have caused die-offs ~~events~~ in numerous fish species~~. and have been detected in a micro-invertebrate (*Diporeia* spp.)~~ (Faisal & Winters, 2011).

x) Genotype IVc

The isolates included in this genotype ~~originate~~ have been detected from finfish from the estuarine waters of New Brunswick and Nova Scotia, Canada (Gagne *et al*., 2007; Pierce & Stepien, 2012; Stepien *et al*., 2015).

xi) Genotype IVd

The isolates included in this genotype ~~originate~~ have been detected in ~~from~~ Iceland where they were identified in wild and farmed lumpfish (*Cyclopterus lumpus*) (Gudmundsdottir *et al*., 2019).

2.1.2. Survival and stability in processed or stored samples

VHSV survival in host tissue is dependent on the conditions for storage. VHSV remains infectious for long time periods while stored frozen in fish tissue. However, VHSV-infected fish at commercial freezing temperatures (–20°C) had a 90% reduction in viral titre after the tissue was thawed (Arkush *et al*., 2006). VHSV is sensitive to enzymatic degradation, environments with high bacterial load and high temperatures (above 28°C). Fresh (unfrozen) muscle tissue from VHSV-infected rainbow trout could transmit infection with VHSV to naïve fish (Oidtmann *et al.,* 2011). VHSV is also tolerant of high salt concentrations such as in brine-treated fish (Skall *et al.,* 2015) or while stored in concentrated ammonium sulphate solution (Pham *et al*., 2018). For optimal retention of VHSV in fish tissue, the sample should be placed in transport medium with antibiotics and kept on ice without freezing and processed within 24 hours after sampling.

2.1.3. Survival and stability outside the host

VHSV survival outside the host is dependent on the physico-chemical conditions of the aqueous medium (Ahne, 1982) and on temperature: the virus survives for longer periods at 4°C compared with 20°C (Parry & Dixon, 1997).

VHSV is significantly more stable in freshwater than saltwater. The virus has been documented to persist in freshwater for 28–35 days at 4°C (Parry & Dixon, 1997) and has been found to be infective for 1 year at 4°C in filtered freshwater (Hawley & Garver, 2008). In raw freshwater at 15°C, the 99.9% inactivation time was 13 days, but in seawater the virus was inactivated within 4 days (Hawley & Garver, 2008). In another study using seawater at 15°C, the infectivity of the virus was reduced by 50% after 10 hours, but could still be recovered after 40 hours (Kocan *et al.,* 2001). There appears to be no consistent correlation between the origin and stability of the virus isolates: freshwater isolates are not always the most stable in freshwater and seawater isolates are not consistently more stable in seawater (Hawley & Garver, 2008).

The virus remains stable for a longer time if sterile organic materials are added to the water, such as ovarian fluids or blood products, such as bovine serum (Kocan *et al.,* 2001). When the sea water was sterilised by autoclaving, or when passed through a 0.22 µm membrane, virus survival was prolonged significantly (60 days at 15°C and 32 days at 20°C), suggesting the bacterial load in the water is an important factor of viral decay.

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with VHSV according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) include:

Family	Scientific name	Common name	Genotype
Ammodytidae	*Ammodytes hexapterus*	Pacific sand lance	IVa
Aralichthyidae	*Paralichthys olivaceus*	Bastard halibut	IVa
Carangidae	*Trachurus mediterraneus*	Mediterranean horse mackerel	Ie
Centrarchidae	*Ambloplites rupestris*	Rock bass	IVb
	Lepomis gibbosus	Pumpkinseed	IVb
	Lepomis macrochirus	Bluegill	IV, IVb
	Micropterus dolomieu	Smallmouth bass	IVb
	Micropterus salmoides	Largemouth bass	IVb
	Pomoxis nigromaculatus	Black crappie	IVb
Clupeidae	*Alosa immaculata*	Pontic shad	Ie
	Sardina pilchardus	Pilchard	ND
	Clupea harengus	Atlantic herring	Ib, III
	Clupea pallasii pallasii	Pacific herring	IVa
	Dorosoma cepedianum	American gizzard shad	IVb
	Sardinops sagax	South American pilchard	IVa
	Sprattus sprattus	European sprat	Ib
Cyclopteridae	*Cyclopterus lumpus*	Lumpfish	IVd
Cyprinidae	*Danio rerio*	Zebra fish	IVa
	Notropis hudsonius	Spottail shiner	IVb
	Notropis atherinoides	Emerald shiner	IVb
	Pimephales notatus	Bluntnose minnow	IVb
	Pimephales promelas	Fathead minnow	IVb
Embiotocidae	*Cymatogaster aggregata*	Shiner perch	IVa
Engraulidae	*Engraulis encrasicolus*	European anchovy	Ie
Esocidae	*Esox lucius*	Northern pike	IVb
	Esox masquinongy	Muskellunge	IVb
Fundulidae	*Fundulus heteroclitus*	Mummichog	IVc
Gadidae	*Gadus macrocephalus*	Pacific cod	IVa
	Gadus morhua	Atlantic cod	Ib, III
	Merlangius merlangus	Whiting	Ie
	Micromesistius poutassou	Blue whiting	Ib, III
	Trisopterus esmarkii	Norway pout	Ib, III

Gasterosteidae	*Gasterosteus aculeatus*	Three-spine stickleback	IVc
Gobiidae	*Neogobius melanostomus*	Round goby	IVb
	Pomatoschistus minutus	Sand goby	Ib
Ictaluridae	*Ictalurus nebulosus*	Brown bullhead	IVb
Labridae	*Centrolabrus exoletus*	Rock cook wrasse	III
	Ctenolabrus rupestris	Goldsinny wrasse	III
	Labrus bergylta	Ballan wrasse	III
	Labrus mixtus	Cuckoo wrasse	III
	Symphodus melops	Corkwing wrasse	III
Lotidae	*Gaidropsarus vulgaris*	Three-bearded rockling	Ie
Moronidae	*Morone americana*	White perch	IVb
	Morone chrysops	White bass	IVb
	Morone saxatilis	Striped bass	IVb, IVc
Mullidae	*Mullus barbatus*	Red mullet	Ie
Osmeridae	*Thaleichthys pacificus*	Eulachon	IVa
Percidae	*Sander vitreus*	Walleye	IVb
	Perca flavescens	Yellow perch	IVb
Petromyzontidae	*Lampetra fluviatilis*	River lamprey	II
Pleuronectidae	*Limanda limanda*	Common dab	Ib
	Platichthys flesus	European flounder	Ib
	Pleuronectes platessus	European plaice	III
Rajidae	*Raja clavata*	Thornback ray	Ie
Salmonidae	*Coregonus artedii*	Lake cisco	IVb
	Coregonus clupeaformis	Lake whitefish	IVb
	Coregonus lavaretus	Common whitefish	Ia
	Oncorhynchus kisutch	Coho salmon	IVa
	Oncorhynchus mykiss	Rainbow trout	Ia-e, III, IVb
	Oncorhynchus mykiss X *Oncorhynchus kisutch* hybrids	Rainbow trout X coho salmon hybrids	Ia
	Oncorhynchus tshawytscha	Chinook salmon	IVa, IVb
	Salmo marmoratus	Marble trout	Ia
	Salmo salar	Atlantic salmon	Ia, Ib, II, III, IVa
	Salmo trutta	Brown trout	Ia, Ib
	Salvelinus namaycush	Lake trout	Ia, IVa, IVb
	Thymallus thymallus	Grayling	I
Scophthalmidae	*Scophthalmus maxima*	Turbot	Ib, III
Sciaenidae	*Aplodinotus grunniens*	Freshwater drum	IVb
Scombridae	*Scomber japonicus*	Pacific chub mackerel	IVa
Soleidae	*Solea senegalensis*	Senegalese sole	III
Uranoscopidae	*Uranoscopus scaber*	Atlantic stargazer	Ie

ND: Not determined.

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to infection with VHSV according to Chapter 1.5 of the *Aquatic Code* include:

Family	Scientific name	Common name	Genotype
Adrianichthydiae	*Oryzias latipes*	Japanese rice fish	IVb
	Oryzias dancena	Marine medaka	IVa
Ammodytidae	*Ammodytes personatus*	Sandeel	Ib
Anguillidae	*Anguilla anguilla*	European eel	III
Argentinidae	*Argentina sphyraena*	Lesser Argentine	Ib
Belonidae	*Belone belone*	Garfish	Ie
Carangidae	*Seriola dumerili*	Greater amberjack	IVa
Catostomidae	*Catostomus commersonii*	White sucker	IVb
	Moxostoma anisurum	Silver redhorse	IVb
	Moxostoma macrolepidotum	Shorthead redhorse	IVb
Centrarchidae	*Pomoxi annuluris*	White crappie	IVb

Clupeidae	*Alosa pseudoharengus*	Alewife	IVb
	~~*Clupea harengus*~~*	~~Atlantic herring~~*	~~IVa~~
Cottidae	*Cottus pollux*	Japanese fluvial sculpin	IVb
Cyprinidae	*Semotilus corporalis*	Fallfish	IVb
	Notemigonus crysoleucas	Golden shiner	IVb
Esocidae	*Esox lucius* X *E. masquinongy* hybrids	Tiger muskellunge (*Esox masquinongy* X *E. lucius* or *E. lucius* X *E. masquinongy*)	IVb
Fundulidae	*Fundulus diaphanus*	Banded killifish	IVb
Gadidae	*Gadiculus argenteus*	Silvery pout	Ib
	Melanogrammus aeglefinus	Haddock	III
	Theragra chalcogramma	Alaska pollock	IVa
	Trisopterus minutus	Poor cod	III
Ictaluridae	*Ictalurus punctatus*	Channel catfish	IVb
Liparidae	*Liparis tessellatus*	Cubed snailfish	IV
Lotidae	*Lota lota*	Burbot	IVb
	Enchelyopus cimbrius	Fourbeard rockling	Ib
Merlucciidae	*Merluccius productus*	North Pacific hake	IVa
Moronidae	*Dicentrarchus labrax*	European sea bass	Ia
Mugilidae	*Mugil cephalus*	Flathead grey mullet	IV
Ophidiidae	*Hoplobrotula armata*	Armoured cusk	IV
Osmeridae	*Hypomesus pretiosus*	Surf smelt	ND
Oxudercidae	*Rhinogobius* sp. (undescribed species)	Yoshinobori	IVb
Percopsidae	*Percopsis omiscomaycus*	Trout perch	IVb
Pleuronectidae	*Glyptocephalus stelleri*	Blackfin flounder	IVa
	Hippoglossus hippoglossus	Atlantic halibut	III
	Reinhardtius hippoglossoides	Greenland halibut	III
Salmonidae	*Oncorhynchus mykiss* X *Salvelinus alpinus* hybrids	Rainbow trout X Arctic charr hybrids	Ia
	Oncorhynchus mykiss X *Salvelinus namaycush* hybrids	Rainbow trout X lake trout hybrids	Ia
	Oncorhynchus mykiss X *Salmo trutta hybrids*	Rainbow trout X brown trout hybrids	Ia
	Salvelinus alpinus	Arctic charr	Ia
	Salvelinus fontinalis	Brook trout	Ie
Sciaenidae	*Larimichthys polyactis*	Yellow croaker	IV
Scorpaenidae	*Scorpaena porcus*	Black scorpionfish	Ie
	Scorpaena izensis	Izu scorpionfish	IV
Scyliorhinidae	*Scyliorhinus torazame*	Claudy catshark	IV
Stromateidae	*Pampus argenteus*	Silver pomfret	IV
Trichiuridae	*Trichiums lepturus*	Largehead hairtail	IV
Triglidae	*Eutrigla gurnardus*	Gray gurnard	III

ND: Not determined.

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following organisms, but an active infection has not been demonstrated: Sablefish (*Anoplopoma fimbria*).

2.2.3. Non-susceptible species

None known.

2.2.4. Likelihood of infection by species, host life stage, population or sub-populations

Rainbow trout is the most susceptible species to VHSV infection with genotype Ia. For VHSV genotypes Ib, II and III, shoaling wild-living species such as Atlantic herring and European sprat ~~(*Sprattus sprattus*)~~ are likely to be the natural hosts, while for genotype IVa, Pacific herring is the natural host. VHSV genotype III has caused disease in farmed turbot and wrasse and genotype IVa in farmed Atlantic salmon, turbot, and ~~olive flounder~~ bastard halibut.

Infection with VHSV may cause disease and mortality in all life stages of susceptible fish. VHSV does not infect fish eggs (Munro & Gregory, 2010).

In surveys of wild marine fish, VHSV has been isolated from most year classes. Few fry have been tested however, as they are usually not caught during the surveys. The highest prevalence of virus in sampled wild populations was found in shoaling fish, such as Atlantic herring, European sprat and Norway pout ~~(*Sprattus sprattus*)~~ (Skall *et al*., 2005a).

2.2.5. Distribution of the pathogen in the host

In fish showing clinical signs, the virus is abundant in all tissues including gill, skin and muscles (Sandlund *et al.,* 2014). Target organs are anterior kidney, heart and spleen, as these are the sites in which virus is most abundant. In chronic stages, virus titres can become high in the brain (Smail & Snow, 2011; Wolf, 1988).

2.2.6. Aquatic animal reservoirs of infection

Some survivors of epizootics will become long-term carriers of the virus. Pacific herring surviving infection with VHSV genotype IVa have transmitted disease to naïve cohabitants (Gross *et al*., 2019). Almost all isolations of VHSV genotype Ib, II and III from free-living fish species are from individuals with no clinical signs of infection with VHSV and with low virus titres (Skall *et al.,* 2005a).

2.2.7. Vectors

VHSV has been isolated from common snapping turtle (*Chelra serpentina*), leech (*Myzobdella lugubris*), northern map turtle (*Grapetemys geographicas*) and water flea (*Moina macrocopa*) and these species ~~are considered~~ may be potential ~~to be~~ vectors for transmission of VHSV ~~rather than true susceptible species~~ (Faisal & Schultz, 2009; Goodwin & Merry, 2011; Ito & Olesen, 2017). VHSV has also been isolated from the amphipods *Hyalellea* spp. and *Diporeia spp*., suggesting that benthic macroinvertebrates may be vectors for VHSV IVb in endemically affected systems. In contrast VHSV was not detected in mussels or sediments in the same water environment (Faisal & Winters 2011; Throckmorton *et al*., 2017). VHSV has also been isolated from leech, *Myzobdella lugubris,* in the Great Lakes ~~but whether the leech or amphipods can transmit VHSV from one fish to another is unknown~~ (Faisal & Schulz, 2009; Faisal & Winters, 2011).

Piscivorous birds may act as VHSV vectors by carrying the virus, for example, on their feet (Olesen & Jorgensen, 1982), or through regurgitation of infected fish (Peters & Neukirch, 1986).

2.3. Disease pattern

2.3.1. Mortality, morbidity and prevalence

Mortality varies, depending on many environmental and physiological conditions, most of which have not been fully determined. The disease is, in general, a cool or cold water disease with highest mortality at temperatures around 9–12°C. Small rainbow trout fry (0.3–3 g) are most susceptible to genotype Ia with mortalities close to 100%, but all sizes of rainbow trout can be affected with mortalities ranging from 5 to 90% (Skall *et al.*, 2004). Immersion infection trials also induced up to 100% mortality in Pacific herring when challenged with genotype IVa (Hershberger *et al.,* 2010). Mortality in free living fish also varies from no observable deaths to severe die-offs. The prevalence of VHSV genotype Ib, II and III varies from 0 to 16.7% in Northern European waters (Skall *et al.*, 2005b).

2.3.2. Clinical signs, including behavioural changes

The occurrence of the following clinical signs is characteristic of infection with VHSV: rapid onset of mortality, lethargy, darkening of the skin, exophthalmia, anaemia (pale gills), haemorrhages at the base of the fins or in the gills, eyes or skin, abnormal swimming such as flashing and spiralling, and a distended abdomen due to oedema in the peritoneal cavity. In rainbow trout, the clinical appearance is typically lethargic dark fish with exophthalmia at the pond shores and the outlet. Characteristically, diseased fish will not attempt to escape when netted.

Some genotypes have specific predominant clinical signs of infection with VHSV. Skin lesions in cod and herring from the Pacific and Atlantic Oceans (including the North Sea), and in haddock from the North Sea, have been described frequently (Jensen & Larsen, 1979; Meyers *et al.,* 1992; Meyers & Winton, 1995; Smail, 2000; Vestergard Jorgensen & Olesen, 1987). In farmed Japanese flounder, an 'anaemic' form (pale gills) of infection with VHSV has also been described (Isshiki *et al.,* 2001).

2.3.3 Gross pathology

Gross pathology includes generalised petechial haemorrhaging in the skin, muscle tissue (especially in dorsal muscles) and internal organs. It is important to examine the dorsal musculature for the presence of petechial bleeding, which is a very common sign of infection with VHSV. The kidney is dark red in the acute phase and can demonstrate severe necrosis in moribund fish. The spleen is moderately swollen. The liver is often pale and mottled. The gastrointestinal tract, especially the hind gut, is pale and devoid of food.

2.3.4. Modes of transmission and life cycle

Transmission primarily occurs horizontally through water, with excretion of virus in the urine, and directly from the skin (Smail & Snow, 2011). Oral transmission was also demonstrated indicating that preying on infected fish and vectors may transfer the disease (Schonherz *et al.* 2012).

Experimentally it has been demonstrated that feeding fresh (unfrozen) muscle tissue from VHSV-infected rainbow trout can transmit VHSV to naïve fish (Oidtmann *et al.,* 2011).

There are no indications or evidence of true vertical transmission of VHSV (Bovo *et al*., 2005a; Munro & Gregory, 2010).

2.3.5. Environmental and management factors

Disease generally occurs at temperatures between 4°C and 14°C. At water temperatures between 15°C and 18°C, the disease generally takes a short course with low levels of mortality.

Low water temperatures (1–5°C) generally result in an extended disease course with low daily mortality but high accumulated mortality. Outbreaks of infection with VHSV occur during all seasons but are most common in spring when water temperatures are rising or fluctuating.

Field observations and experimental studies suggest that warmer water temperatures greatly reduce or inhibit transmission. Natural outbreaks of infection with VHSV are not observed at water temperatures greater than 18°C. In challenge trials, fish exposed to VHSV and reared at temperatures below 15°C displayed high mortality whereas those infected and reared at 20°C did not (Arkush *et al.,* 2006; Castric & de Kinkelin, 1984). For more detailed reviews, see Wolf (1988) and Smail & Snow (2011).

2.3.6. Geographical distribution

~~Until the late 1980s, VHSV was considered to be restricted to farmed rainbow trout in continental Europe, with the occasional isolation from a restricted number of other freshwater fish species (e.g. brown trout, pike [Meier & Jorgensen, 1980; Schlotfeldt & Ahne, 1988]). With the detection and isolation of VHSV from Pacific salmon off the Pacific North American coast in the late 1980s, subsequent studies have demonstrated that infection with VHSV occurs in numerous farmed and wild fish species along the Pacific and Atlantic North American coast (Skall *et al*., 2005), in the Great Lakes area of North America (Thompson *et al*., 2011), the seas around the UK (Skall *et al*., 2005), the Baltic Sea, Skagerrak and Kattegat (Skall *et al*., 2005), in the waters around Japan (Skall *et al*., 2005), and in the Black Sea area, with the distinct genotype Ie (Nishizawa *et al*., 2006).~~

Infection with VHSV ~~in farmed rainbow trout~~ has been reported from countries in Europe, North Amercia and North Asia. Some countries in these regions have declared freedom from infection with VHSV. ~~almost all European and Middle East countries and from China (People's Rep. of) and Russia. However, a number of countries in Europe, such as Denmark, Ireland, Norway, Sweden and UK, are officially declared free of infection with VHSV. Infection with VHSV~~ The disease has never been reported from the Southern Hemisphere.

For recent information on distribution at the country level consult the WAHIS interface (https://www.oie.int/wahis_2/public/wahid.php/Wahidhome/Home/index/newlang/en).

2.4. Biosecurity and disease control strategies

2.4.1. Vaccination

Although research on vaccine development for VHSV has been ongoing for more than four decades, the only ~~a~~ commercial vaccine ~~is not yet~~ available is against VHSV genotype IVa for bastard halibut in Korea. Candidate vaccines have included killed vaccines, attenuated live vaccines, a recombinant vaccine in prokaryotic and eukaryotic expression systems, and DNA-based vaccines. For a review see Lorenzen & LaPatra (2005). No vaccines currently affect the sensitivity and specificity of infection with VHSV diagnostics.

2.4.2. Chemotherapy including blocking agents

No therapies are currently available.

2.4.3. Immunostimulation

Several immunostimulants, such as yeast-derived beta-glucans, IL-1β-derived peptides, and probiotics have been assessed for enhancing protection against infection with VHSV (Peddie *et al*., 2003). Several researchers report positive effects, but no immunostimulant directed specifically at enhanced resistance to infection with VHSV is available. Furthermore, it remains unknown as to whether their use can affect sensitivity and specificity of ~~infection with~~ VHSV assays~~diagnostics~~.

2.4.4. Breeding resistant strains

Additive genetic variation in rainbow trout ~~has been detected~~ for resistance to infection with VHSV has been demonstrated (Dorson *et al*., 1995; Henryon *et al*., 2002a; 2002b). In a study by Henryon *et al*. (2005), the heritability of resistance to VHS was 0.11 for time to death on a logarithmic timescale. Identification of a major quantitative trait loci (QTL) for VHSV resistance in rainbow trout may pave the way for genetic selection for VHSV resistant fish (Verrier *et al.,* 2013), however, no resistant rainbow trout strains are ~~yet~~ commercially available.

2.4.5. Inactivation methods

VHSV is sensitive to a number of common disinfectants (e.g. UV, chlorine, iodophore, sodium hypochlorite), to temperatures above 30°C, to bacterial degradation in sediments and enzymatic activity in decomposing fish. For a review see Bovo *et al*., 2005b.

2.4.6. Disinfection of eggs and larvae

Disinfection of newly fertilised or eyed ~~and green~~ eggs is an efficient and cost-effective preven~~ta~~tive measure for stopping the spread of the disease in salmonids (for the recommended protocol see Chapter 4.4. of the *Aquatic Code*).

2.4.7. General husbandry

Poor water quality, high fish density, high feeding rate, infection with other diseases such as proliferative kidney disease, ichthyophthiriasis, bacterial kidney disease, etc. can influence the course and severity of infection with VHSV. In general, an increase in temperature, restricted feeding, reduced fish density and restricted handling may reduce mortality. In endemically infected farms, stocking with naïve fry is usually done ~~at as high~~ when the water temperature~~s~~ is at near maximum levels~~as possible~~.

3. Specimen selection, sample collection, transportation and handling

3.1. Selection of populations and individual specimens

Clinical inspections should be carried out during a period when the water temperature is below 14°C or whenever the water temperature is likely to reach its lowest annual point. All production units (ponds, tanks, net-cages, etc.) should be inspected for the presence of dead, weak or abnormally behaving fish. Particular attention should be paid to the water outlet area where weak fish tend to accumulate due to the water current.

Fish to be sampled are selected as follows:

i) For genotype I, in farms where rainbow trout are present, ~~only~~ fish of that species should be selected for sampling. If rainbow trout are not present, the sample should be obtained from fish of all other VHSV-susceptible species present (as listed in Table~~s~~ 2.1) ~~and~~ or from species with incomplete evidence for susceptibility (as listed in Table 2.2). However, the species should be proportionally represented in the sample. For other genotypes (II, III, and IV), species of known susceptibility to the genotype in question should be sampled.

ii) Susceptible species should be sampled following risk-based criteria for targeted selection of populations with a history of abnormal mortality or potential exposure events (e.g. via untreated surface water, wild harvest or introduction of stocks of unknown risk status).

iii) If more than one water source is used for fish production, fish from all water sources should be included in the sample.

3.2. Selection of organs or tissues

The optimal tissues ~~material to be examined is~~ are spleen, anterior kidney, heart and encephalon. When sampling broodstock, ~~In some cases,~~ ovarian fluid and milt can be taken ~~must 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 anal pore ~~gut opening~~. If a sample consists of whole fish with a body length between 4 cm and 6 cm, the viscera including kidney should be collected. For larger size fish, kidney, spleen, heart and encephalon, and ovarian fluid from brood fish at the time of spawning should be the tissues to be sampled.

3.3. Samples or tissues not suitable for pathogen detection

VHSV is very sensitive to enzymatic degradation, therefore, sampling tissues with high enzymatic activities, such as viscera and liver, or large numbers of contaminating bacteria, such as the intestine or skin, should be avoided. Preservatives and fixatives, such as RNAlater and formaldehyde can be toxic to tissue culture cells such as epithelioma papulosum cyprini (EPC) and fathead minnow (FHM), and can impact molecular detection methods (Auinger *et al.,* 2008; Pham *et al.,* 2018).

3.4. Non-lethal sampling

Fin and gill biopsies were shown to be effective nonlethal samples for detection of VHSV genotype IVb (Cornwell *et al.,* 2013) in clinically diseased fish and nested reverse-transcription polymerase chain reaction (RT-PCR) on blood samples from infected fish was also shown to be effective ~~efficient~~ for VHSV detection (Lopez-Vazquez *et al.,* 2006a). In the case of brood fish, ovarian fluid and milt can be used for testing as an alternative to lethal testing. However, ~~no~~ non-lethal sampling~~s~~ methods have not been fully validated for detection of all VHSV genotypes ~~and are therefore not prescribed in this chapter~~.

3.5. Preservation of samples for submission

For guidance on sample preservation methods for the intended test methods, see Chapter 2.3.0.

3.5.1. Samples for pathogen isolation

The success of pathogen isolation and results of bioassay depend ~~strongly~~ heavily on the quality of samples (time since collection and time in storage). Fresh specimens should be kept on ice and preferably sent to the laboratory within 24 hours of collection. Alternate storage methods should be used only after consultation with the receiving laboratory.

Before transfer to the laboratory, pieces of the organs to be examined should be removed from the fish with sterile dissection tools and transferred to sterile plastic tubes containing at least 4 ml transport medium, i.e. cell culture medium with 10% fetal calf serum (FCS) and antibiotics. The combination of 200 International Units (IU) penicillin, 200 µg streptomycin, and 200 µg kanamycin per ml are recommended, although other antibiotics of proven efficiency may also be used. The tissue in each sample should be larger than the analytical unit size required for initial laboratory testing (e.g. between 0.5 and 2 g) and taken in duplicate if retesting may be required.

Tubes containing fish tissues in transport medium for cell cultivation should be placed in insulated containers, such as thick-walled polystyrene boxes, together with sufficient ice or an alternative cooling medium with the similar cooling effect to ensure chilling of the samples during transportation to the laboratory. However, freezing of the samples should be avoided. The temperature of a sample during transit must never exceed 10°C and ice must still be present in the transport box at receipt or one or more freeze blocks must still be partly or completely frozen.

Whole fish may be sent to the laboratory if the temperature requirements referred to in the first paragraph during transportation can be fulfilled. Whole fish should be wrapped up in paper with absorptive capacity and enclosed in a plastic bag. Live fish may also be transported to the laboratory. All packaging and labelling must be performed in accordance with ~~present~~ current national and international transport regulations, as appropriate.

The virological examination for isolation in cell culture should be started as soon as possible and no later than 48 hours after the collection of the samples. In exceptional cases, the virological examination may be started at the latest within 72 hours after the collection of the material, provided that the material to be examined is protected by a transport medium and that the temperature requirements during transportation can be fulfilled.

3.5.2. Preservation of samples for molecular detection

Samples can be taken from the fish in accordance with the procedure described in Section 3.5.1., using a sterile instrument, and transferred to a sterile plastic tube containing transport medium.

Alternatively, samples may be placed in at least five volumes of RNA stabilisation reagents according to the recommendation from the manufacturers. Samples in RNA stabilising reagents can be shipped on ice or at room temperature if transport time does not exceed 24 hours.

Whole fish may also be sent to the laboratory (see Section 3.5.1.).

Samples may also be frozen at –80°C and kept frozen until assayed (Siah *et al.*, 2014).

3.5.3. Samples for histopathology, immunohistochemistry or *in-situ* hybridisation

Tissue samples for histopathology should be fixed in 10% neutral buffered formalin immediately after collection. The recommended ratio of fixative to tissue is 10:1. To avoid excessive cross-linking, tissue should be transferred to ethanol after 24 hours if methods other than histopathology are used e.g. *in-situ* hybridisation.

3.5.4. Fixed samples for electron microscopy

~~Sampling for electron microscopy should be done according to standard procedures (for an example, see Chapter 2.2.9 *Infection with yellow head virus genotype 1*).~~ Sampling for electron microscopy is not relevant for diagnostic purposes. Samples for electron microscopy are not routinely required and are collected only when it is considered beneficial to facilitate further diagnostic investigation. A 2 mm cubed section from each of the appropriate organs described in section 3.2 should be fixed in glutaraldehyde; the recommended ratio of fixative to tissue is 10:1.

3.5.5. Samples for other tests

If samples are processed for ELISA or other immunochemical assays, the procedures described in Section 3.5.1. for pathogen isolation should be followed.

3.6. Pooling of samples

The effect of pooling on diagnostic sensitivity has not been evaluated, therefore, larger fish should be processed and tested individually. However, samples, especially fry or specimens up to 0.5 g, can be pooled to obtain enough material for virus isolation or molecular detection.

4. Diagnostic methods

The methods currently available for identifying infection that can be used in i) surveillance of apparently healthy populations), ii) presumptive and iii) confirmatory diagnostic purposes are listed in Table 4.1. by life stage. The designations used in the Table indicate:

Key:

+++ =	Recommended method(s) validated for the purpose shown and usually to stage 3 of the OIE Validation Pathway;
++ =	Suitable method(s) but may need further validation;
+ =	May be used in some situations, but cost, reliability, lack of validation or other factors severely limits its application;
Shaded boxes =	Not appropriate for this purpose.

The selection of a test for a given purpose depends on the analytical and diagnostic sensitivities and specificities, and repeatability and reproducibility. OIE Reference Laboratories welcome feedback on diagnostic performance for assays, in particular PCR methods, for factors affecting assay analytical sensitivity or analytical specificity, such as tissue components inhibiting amplification, presence of nonspecific or uncertain bands, etc., and any assays that are in the +++ category.

Table 4.1. OIE recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals

Method	A. Surveillance of apparently healthy animals				B. Presumptive diagnosis of clinically affected animals				C. 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
Wet mounts												
Immunohistopathology[3]									++	~~2~~ ++	~~2~~ ++	2
Histopathology[3]						++	++	1				
Cell culture	+++	+++	+++	3	+++	+++	+++	3	+++	+++	+++	3
Real-time PCR	+++	+++	+++	3	+++	+++	+++	3	+++	+++	+++	3
Conventional RT-PCR	++	++	++	3	+++	++	+++	3	+++	++	+++	3
Amplicon sequencing[4]									+++	+++	+++	3
***In-situ* hybridisation**												
Bioassay												
LAMP												
Ab-ELISA		+	++	2								
Ag-ELISA									+[5]	++[5]	++[5]	1
IFAT					++	++	++	2	++[5]	++[5]	++[5]	2
Serum neutralisation for Ab detection		+	++	2								

LV = level of validation, refers to the stage of validation in the OIE Pathway (chapter 1.1.2); RT-PCR = reverse-transcription polymerase chain reaction; LAMP = loop-mediated isothermal amplification; Ab- or Ag-ELISA = antibody or antigen enzyme-linked immunosorbent assay, respectively; IFAT = indirect fluorescent antibody test;
[1]For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). [2]Early and juvenile life stages have been defined in Section 2.2.4. [3]Histopathology and cytopathology can be validated if the results from different operators has been statistically compared. [4]Sequencing of the PCR product. [5]only for identification of cultured pathogen.
Shading indicates the test is inappropriate or should not be used for this purpose.

4.1. Wet mounts

Not relevant.

4.2. Histopathology and cytopathology

The kidney, liver and spleen show extensive focal necrosis and degeneration – cytoplasmic vacuoles, pyknosis, karyolysis, and lymphocytic invasion. While the skeletal muscle does not appear to be a site of infection, erythrocytes can accumulate in the skeletal muscle bundles and fibres without causing damage to the muscle *per se* (Evensen *et al.,* 1994).

4.3. Cell or artificial media culture for isolation

The recommended cell lines for VHSV detection are bluegill fry (BF-2), epithelioma papulosum cyprini (EPC) or fathead minnow (FHM). Susceptibility of a cell line to VHSV infection will depend on a range of parameters, including cell-line lineage or viral strain differences. Generally, VHSV isolates belonging to either genotypes I, II, or III culture best on BF-2 (Lorenzen *et al*., 1999), while genotype IV isolates culture best on the EPC cell line (US Department of the Interior, 2007).

4.3.1. Cell lines

Cell lines should be monitored regularly (e.g. every 6 months) to ensure that susceptibility to targeted pathogens has not changed.

Cells are grown at 20–24°C in a suitable medium, e.g. Eagle's minimal essential medium (MEM) (or modifications thereof) with a supplement of 10% fetal bovine serum (FBS) and antibiotics in standard concentrations. When the cells are cultivated in closed vials, it is recommended to buffer the medium with bicarbonate. The medium used for cultivation of cells in open units may be buffered with Tris/HCl (23 mM) and Na-bicarbonate (6 mM), or with HEPES-buffered medium (HEPES=N-2-hydroxyethyl-piperazine-N-2-ethanesulphonic acid). The pH must be maintained at 7.6 ± 0.2. Cell cultures to be used for inoculation with tissue material should be young (4–48 hours old) and actively growing (not confluent) at inoculation. Cell susceptibility can be enhanced by reducing the amount of FBS to 2%. Pre-treatment of cells with 7% (w/v) PEG-20,000 solution (10–15 µl/cm^2) 15–30 minutes prior to sample inoculation has also been shown to increase detection of VHSV in culture (Batts *et al.,* 1991).

4.3.2. Sample preparation and inoculation

i) **Note:** Tissue and fluid samples should be kept cool throughout sample preparation procedures. Homogenise tissue samples using mortar and pestle~~, stomacher, polytron or equivalent~~ or a tissue homogeniser. A small volume of media (MEM-4 or HBSS [Hank's balanced salt solution] + antibiotics) may be needed to achieve complete homogenisation.

ii) Adjust the volume of media to a final ratio of 10:1 (media:tissue) and mix thoroughly. For fluid samples adjust the volume of media to a final ratio of 1:1.

iii) Centrifuge the homogenate or fluid samples at 2000–4000 ***g*** for 15 minutes at 2–5°C.

iv) Remove the supernatant and pass through a 0.45 µM membrane filter (if available) or treat for either 4 hours at 15°C or overnight at 4°C with antibiotics, e.g. gentamicin 1 mg ml^{-1}.

    If the sample cannot be inoculated within 48 hours after collection, the supernatant may be stored at –80°C provided virological examination is carried out within 14 days.

v) If samples originate from an area where infectious pancreatic necrosis virus (IPNV) is present, supernatants may be treated with IPNV antiserum. Mix the supernatant with equal parts of a suitably diluted pool of antisera to the indigenous serotypes of IPNV and incubate for a minimum of one hour at 15°C or up to 18 hours at 4°C. The titre of the antiserum must be at least 1/2000 in a 50% plaque neutralisation test.

Treatment of all inocula with antiserum to IPNV ~~(a virus that in some parts of Europe occurs in 50% of fish samples)~~ aims at preventing cytopathic effect (CPE) caused by IPNV from developing in inoculated cell cultures. This will reduce the duration of the virological examination as well as the number of cases in which occurrence of CPE would have to be considered potentially indicative of VHSV. When samples come from production units that are considered free from infection with IPNV, treatment of inocula with antiserum to IPNV may be omitted.

vi) Samples are inoculated into cell cultures in at least two dilutions, i.e. the primary dilution and a 1:10 dilution thereof, resulting in final dilutions of tissue material in cell culture medium of 1:100 and 1:1000, respectively. The ratio between inoculum size and volume of cell culture medium should be about 1:10. For each dilution and each cell line, a minimum of about 2 cm^2 cell area, corresponding to one well in a 24-well cell culture tray, has to be used. Use of cell culture trays is recommended, but other units of similar or with larger growth area are also acceptable.

vii) Inoculated cell cultures are incubated at 15°C for 7–10 days. Using a microscope with 40–150× magnification, cultures should be inspected for toxicity the day after inoculation, particularly if supernatant was not filtered in step iv. The use of a phase-contrast microscope is recommended.

viii) Monitor the cells regularly (2–3 times a week) for the presence of CPE.

If CPE is observed, virus identification is required using tests recommended in Section 6. If no CPE is observed after the primary incubation period, subcultivation is performed.

Subcultivation

i) Remove cell culture supernatant from the primary culture and inoculate a newly (<48 hours) seeded cell culture plate.

ii) Incubate inoculated plates at 15°C and monitor for 7–10 days as described above.

If CPE is observed, virus identification is required using tests recommended in Section 6. If no CPE is observed after the primary incubation period or subcultivation, the sample is negative.

4.4. Nucleic acid amplification

Use of molecular tests (RT-PCR and real-time RT-PCR) is common because of their rapidity, sensitivity and specificity. Real-time RT-PCR tests are generally more sensitive than conventional RT-PCR tests. These tests for virus detection and identification during the acute stage of disease has been justified for a number of years. ~~At~~ In the acute stage of infection, the sensitivity of some RT-PCR (Kim *et al.,* 2018) and real-time RT-PCR tests (Garver *et al.,* 2011; Jonstrup *et al.,* 2013) is comparable to detection by cell culture and subsequent identification. The molecular methods described in this chapter are all targeting the ~~N~~nucleoprotein gene, as it is the highest transcribed gene in the VHSV genome (Chico *et al.,* 2006).

Recently, a novel one-step RT-PCR test was developed and validated (Kim *et al.,* 2018) to be used instead of the previously recommended conventional RT-PCR for detecting VHSV. This novel assay has a higher sensitivity detecting all VHSV genotypes, and outperforms the old method, particularly in detecting genotype IV.

For detecting all genotypes of VHSV with real-time RT-PCR, the ~~one-step~~ methods of Jonstrup *et al*. (2013) and Garver *et al.* (2011) have ~~has~~ been validated to stage 3 ~~validated~~, showing a sensitivity similar to detection by cell culture. These~~This~~ methods~~,~~ have ~~having~~ high analytical and diagnostic sensitivity and specificity, and ~~has been shown to be highly~~ are robust across laboratories (Garver *et al.,* 2011; Jonstrup *et al.,* 2013; Warg *et al.,* 2014a; 2014b).

The following controls should be run with each assay: negative extraction control; positive control; no template control; internal PCR control.

4.4.1. Real-time RT-PCR

Total RNA can be purified from: aliquots of cell culture medium from infected monolayer cells; or tissue/organs homogenised in MEM specified in Section 4.3.1, tissue samples in RNA stabilising reagent, fresh or frozen tissue samples, ovarian fluid.

In the case of culture medium from infected monolayer cells, or in tissue homogenised in MEM, aliquots should be centrifuged at 1000 ***g*** for 5 minutes to remove cell debris.

One-step (Jonstrup *et al.,* 2013) and two-step (Garver *et al.,* 2011) real-time RT-PCR assays targeting the nucleoprotein gene of VHSV have been stage 3 validated and are described herein.

Positive and negative controls should be included with each stage of the assay: extraction, reverse-transcription (two-step assay only) and real-time PCR. An internal (endogenous) PCR control can be included however given the large number of fish species susceptible to infection with VHSV, the selection of an internal control is not trivial. If an endogenous control is to be used, primers and probes have to be designed, optimised and validated for each fish species to be tested.

Total RNA from infected cells and/or tissues is extracted using a phase-separation method (e.g. phenol-chloroform or Trizol) or by use of a commercially available RNA isolation kit used according to the manufacturer's instructions.

One-step real-time RT-PCR

In one-step RT-PCR gene-specific primers are used both to generate a cDNA transcript and for real-time PCR. Both reactions occur in the same tube, which minimises the probability of contamination. The one-step real-time RT-PCR amplification can be performed using forward primer 5'-AAA-CTC-GCA-GGA-TGT-GTG-CGT-CC-3', reverse primer: 5'-TCT-GCG-ATC-TCA-GTC-AGG-ATG-AA-3', and FAM-labelled probe: 6'-FAM-TAG-AGG-GCC-TTG-GTG-ATC-TTC-TG-BHQ1. Primers are used at a final concentration of 900 nM and the final probe concentration is 250 nM. 5 µl of extracted RNA (50 ng–2 ug) is added to each 25 µl PCR reaction. The assay was validated using Quantitect Probe RT-PCR kit (Qiagen, Germany) following the manufacturer's instructions and is recommended as other one-step kits have demonstrated reduced sensitivity (Jonstrup *et al.,* 2013). Thermal cycling conditions are 50°C for 30 minutes, 95°C for 15 minutes, 40 cycles of 94°C for 15 seconds, 60°C for 40 seconds, 72°C for 20 seconds.

Two-step real-time RT-PCR

i) Step 1: Reverse-transcription

Extracted RNA is reverse transcribed non-discriminately into cDNA using random primers. The cDNA synthesis reactions and cycling conditions are best performed using manufacturer's instructions for commercially available kits which have been extensively tested with a variety of RNA templates, including GC- and AU-rich targets and RNAse expressed at low levels.

ii) Step 2: Real-time PCR

The TaqMan real-time PCR assay uses forward primer 5'-ATG-AGG-CAG-GTG-TCG-GAG-G-3', reverse primer 5'-TGT-AGT-AGG-ACT-CTC-CCA-GCA-TCC and FAM-labelled probe 5'-6FAM-TAC-GCC-ATC-ATG-ATG-AGT-MGBNFQ-3'. Primers are used at a final concentration of 600 nM, and the final concentration of the probe is 200 nM. 2.5 µl of cDNA product is added to each 25 µl PCR reaction. Thermal cycling conditions are 50°C for 2 minutes, 95°C for 10 minutes followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute.

A sample is negative if no Ct (threshold cycle) is recorded, while samples with a Ct are considered positive for VHSV. Cut-off value depends on the set-up in each laboratory but is usually set at Ct ≥ 40).

4.4.2. Conventional PCR

RNA isolation is done as in Section 4.4.1. Positive and negative controls should be run with each stage of the assays: extraction, RT-PCR and second round PCR. Due to the sensitive nature of PCR-based assays it is highly recommended that master mix, template addition and PCR amplification occur in designated hoods or spatially separated areas.

A one-step RT-PCR should be performed as described by Kim *et al.* (2018) with 3F2R primer set: forward primers (3F, 5'-(GGG-ACA-GGA-ATG-ACC-ATG-AT-3') and reverse primer (2R, (5'-TCT-GTC-ACC-TTG-ATC–CCC-TCC-AG-3') targeting a 319 nt region in the nucleoprotein gene (positions 658–977).

The RT-PCR can be performed using, e.g. Qiagen OneStep RT-PCR System (Qiagen, Germany) or similar kit, according to the manufacturer's instructions. Briefly, the reaction mixture is adjusted to a final volume of 25 µl including 5 µl of extracted viral RNA, 5 µl 5 × One Step RT-PCR Buffer containing 12.5 mM $MgCl_2$ (final concentration 2.5 mM), 10 pM of each primer, and 1 µl of enzyme mix.

The following cycles are recommended: 50°C for 30 minutes, 95°C for 15 minutes, 35 cycles at 94°C for 30 seconds, 60°C for 30 seconds, and 68°C for 60 seconds. Subsequently, the reaction is held at 68°C for 7 minutes.

4.4.3. Other nucleic acid amplification methods

To date, no other nucleic acid amplification method capable of universal VHSV detection has been sufficiently validated.

4.5. Amplicon sequencing

Nucleotide sequencing of the glycoprotein gene is commonly used for identification of genetic strains and for epidemiological study and is recommended as one of the final steps for confirmatory diagnosis. There are several conventional PCR assays available that amplify the central (669 nt) or full (1524 nt) glycoprotein gene coding sequence, but there are limited validation data. The glycoprotein gene can be amplified by conventional PCR using the primer sets and concentrations listed in Table 4.2. The reverse transcription and subsequent PCR amplification can be done using a kit designed for that purpose according to manufacturing instructions.

Table 4.2. *Primer sets for the conventional PCR*

Primer	Sequence (5'–3')	Product size (bp)	Final primer concentration	Reference
GB+	GTC-GAA-GAA-GAG-ATA-GGC	1757	0.6 µM	Einer-Jensen *et al.,* 2004 Gudmundsdottir *et al.,* 2019
GB-	GTT-GGG-TCG-CCA-TGT-TTC-T		0.6 µM	
G330+	ACT-ACC-TAC-ACA-GAG-TGA-C	914	0.2 µM	Garver *et al*., 2013
G1243-	CAA-TTT-GTC-CCC-GAA-TAT-CAT		0.2 µM	
G422+	TCC-CGT-CAA-GAG-GCC-AC	669	0.2 µM	
G1179-	TTC-CAG-GTG-TTG-TTT-ACC-G		0.2 µM	

4.6. *In-situ* hybridisation

Not relevant in relation to primary diagnosis and surveillance of infection with VHSV.

4.7. Immunohistochemistry

Immunohistochemistry reveals VHSV-positive endothelial cells, primarily in the vascular system (Evensen *et al*., 1994). Specific polyclonal and monoclonal antibodies for immunohistochemistry are commercially available.

4.8. Bioassay

Not relevant in relation to primary diagnostics and surveillance of infection with VHSV.

4.9. Antibody- or antigen-based detection methods

Antibody- and antigen-based detection methods should not be used as a method of screening healthy populations~~method~~.

4.9.1. Antigen enzyme-linked immunosorbent assay (ELISA)

i) Coat the wells of microplates designed for enzyme-linked immunosorbent assays (ELISAs) with appropriate dilutions of protein-A purified immunoglobulins (Ig) from rabbit anti sera against VHSV in carbonate buffer, pH 9.6 (50 µl well^{-1}).

ii) Incubate overnight at 4°C.

iii) Rinse in phosphate-buffered saline (PBS) containing 0.05% Tween-20 (PBST).

iv) Add 1% Triton X-100 to the virus suspension to be identified.

v) Dispense 50 µl well^{-1} of two- or four-step dilutions (in PBST containing 1% bovine serum albumin) of the virus to be identified and of VHSV control virus, as well as a negative control (e.g. infectious haematopoietic necrosis virus [IHNV]), and allow to react with the coated antibody to VHSV for 1 hour at 37°C.

vi) Rinse in PBST.

vii) Add to the wells monoclonal antibodies to VHSV N protein (IP5B11) 50 µl well^{-1}.

viii) Incubate for 1 hour at 37°C.

ix) Rinse in PBST.

x) Add to the wells (50 µl well^{-1}) horseradish peroxidase (HRP)-conjugated monoclonal anti-mouse antibodies.

xi) Incubate for 1 hour at 37°C.

xii) Rinse in PBST.

xiii) Visualise the reaction using TMB (3,3',5,5'-tetramethylbenzidine) and measure the absorbance at a wavelength of 450 nm.

The above ELISA version is given as an example. Other ELISA versions of demonstrated performance may be used instead.

For positive controls, use cell culture supernatant from cultures inoculated with known VHSV isolate.

For negative controls, use cell culture supernatant from same cell line inoculated with heterologous virus (e.g. IHNV) or from non-infected culture.

4.9.2. Indirect fluorescent antibody test (IFAT)

i) Prepare monolayers of cells in 2 cm^2 wells of cell culture plastic plates or on cover-slips to reach around 80% confluence, which is usually achieved within 24 hours of incubation at 22°C (seed six cell monolayers per virus isolate to be identified, plus two for positive and two for negative controls). The FCS content of the cell culture medium can be reduced to 2–4%. If numerous virus isolates have to be identified, the use of Terasaki plates is strongly recommended.

ii) When the cell monolayers are ready for infection, i.e. on the same day or on the day after seeding, inoculate the virus suspensions to be identified by making tenfold dilution steps directly in the cell culture wells or flasks.

iii) Dilute the control virus suspension of VHSV in a similar way, in order to obtain a virus titre of about 5000–10,000 plaque-forming units (PFU) ml^{-1} in the cell culture medium.

iv) Incubate at 15°C for 24 hours.

v) Remove the cell culture medium, rinse once with 0.01 M PBS, pH 7.2, then three times briefly with a cold mixture of acetone 30% and ethanol 70% (v/v) (stored at –20°C).

vi) Let the fixative act for 15 minutes. A volume of 0.5 ml is adequate for 2 cm^2 of cell monolayer.

vii) Allow the cell monolayers to air-dry for at least 30 minutes and process immediately or freeze at –20°C.

viii) Prepare a solution of purified VHSV antibody or serum in 0.01 M PBST, pH 7.2, at the appropriate dilution (which has been established previously or is given by the reagent supplier).

ix) Rehydrate the dried cell monolayers by using four rinsing steps with the PBST solution and remove this buffer completely after the last rinse.

x) Treat the cell monolayers with the antibody solution for 1 hour at 37°C in a humid chamber and do not allow evaporation to occur, e.g. by adding a piece of wet cotton in the humid chamber. The volume of solution to be used is 0.25 ml per ~~/~~2 cm^2 well~~$^{-1}$~~.

xi) Rinse four times with PBST as above.

xii) Treat the cell monolayers for 1 hour at 37°C with a solution of fluorescein isothiocyanate (FITC)- or tetramethylrhodamine-5-(and-6-) isothiocyanate (TRITC)-conjugated antibody to the immunoglobulin used as the primary antibody and prepared according to the instructions of the supplier. These conjugated antibodies are most often rabbit or goat antibodies.

xiii) Rinse four times with PBST.

xiv) Examine the treated cell monolayers on plastic plates immediately, or mount the cover-slips using, for example glycerol saline, pH 8.5 prior to microscopic observation.

xv) Examine under incident UV light using a microscope with ×10 eye pieces and ×20–40 objective lens having numerical aperture >0.65 and >1.3 respectively. Positive and negative controls must yield the expected results prior to any other observation.

Other IFAT or immunocytochemical (alkaline phosphatase or peroxidase) techniques of demonstrated performance may be used instead.

Always include positive control such as wells or coverslip with cells infected with a known VHSV isolate.

4.10. Other ~~serological~~ methods

4.10.1. Neutralisation test

i) Collect the culture medium of the cell monolayers exhibiting CPE and centrifuge it at 2000 ***g*** for 15 minutes at 4°C, or filter through a 0.45 µm (or 450 nm) pore membrane to remove cell debris.

ii) Dilute virus-containing medium from 10^{-2} to 10^{-4}.

iii) Mix aliquots (for example 200 µl) of each dilution with equal volumes of a VHSV antibody solution and, likewise, treat aliquots of each virus dilution with cell culture medium. The neutralising antibody [NAb] solution must have a 50% plaque reduction titre of at least 2000.

iv) In parallel, another neutralisation test must be performed against a homologous virus strain (positive neutralisation test).

v) If required, a similar neutralisation test may be performed using antibodies to IPNV.

vi) Incubate all the mixtures at 15°C for 1 hour.

vii) Transfer aliquots of each of the above mixtures on to 24–48 hour-old monolayers, overlaid with cell culture medium containing 10% FCS (inoculate two wells per dilution), and incubate at 15°C; 24- or 12-well cell culture plates are suitable for this purpose, using a 50 µl inoculum.

viii) Check the cell cultures for the onset of CPE and read the result as soon as it occurs in non-neutralised controls (cell monolayers being protected in positive neutralisation controls). Results are recorded either after a simple microscopic examination (phase contrast preferable) or after discarding the cell culture medium and staining cell monolayers with a solution of 1% crystal violet in 20% ethanol.

ix) The tested virus is identified as VHSV when CPE is prevented or noticeably delayed in the cell cultures that received the virus suspension treated with the VHSV-specific antibody, whereas CPE is evident in all other cell cultures.

x) In the absence of any neutralisation by NAb to VHSV, it is mandatory to conduct an RT-PCR, an ELISA or IFAT, using the suspect sample. Some cases of antigenic drift of surface antigen have been observed, resulting in occasional failure of the neutralisation test using NAb to VHSV.

Other neutralisation tests of demonstrated performance may be used instead.

5. Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations

Virus isolation, real-time RT-PCR and conventional PCR are the recommended tests for surveillance to demonstrate freedom of disease in apparently healthy population.

6. Corroborative diagnostic criteria

This section only addresses the diagnostic test results for detection of infection in the absence (Section 6.1.) or in the presence of clinical signs (Section 6.2.) but does not evaluate whether the infectious agent is the cause of the clinical event.

The case definitions for a suspect and confirmed case have been developed to support decision making related to trade and confirmation of disease status at the country, zone or compartment level. Case definitions for disease confirmation in endemically affected areas may be less stringent. It is recommended that all samples that yield suspect positive test results in an otherwise pathogen-free country or zone or compartment should be referred immediately to the OIE Reference Laboratory for confirmation, whether or not clinical signs are associated with the case. If a laboratory does not have the capacity to undertake the necessary diagnostic tests it should seek advice from the appropriate OIE Reference Laboratory.

6.1. Apparently healthy animals or animals of unknown health status[4]

Apparently healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link to an infected population. Geographical proximity to, or movement of animals or animal products or equipment, etc., from a known infected population equate to an epidemiological link. Alternatively, healthy populations are sampled in surveys to demonstrate disease freedom.

6.1.1. Definition of suspect case in apparently healthy animals

The presence of infection with VHSV shall be suspected if at least one of the following criteria is met:

i) VHSV-typical CPE in cell cultures ~~before confirmation~~;

ii) A positive result from a real-time PCR assay;

iii) A positive result from a conventional PCR assay;

iv) Detection of antibodies (by Ab-ELISA or serum neutralisation in adults only).

[4] For example transboundary *commodities.*

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with VHSV is considered to be confirmed if, in addition to the criteria in Section 6.1.1., one or more of the following criteria is met:

~~i)~~ ~~VHSV isolation in cell culture followed by virus identification by conventional RT-PCR, and by sequencing of the amplicon;~~

~~ii)~~ i) VHSV isolation in cell culture, followed by virus identification by real-time RT-PCR, Ag-ELISA, or IFAT and detection of VHSV in tissue preparations by conventional RT-PCR and sequencing of the amplicon;

~~iii)~~ ii) VHSV isolation in cell culture, followed by virus identification by real-time RT-PCR, Ag-ELISA, or IFAT and detection of VHSV in tissue preparations by real-time RT-PCR;

~~iv)~~ iii) Detection of VHSV in tissue preparations by real-time RT-PCR, and by a conventional RT-PCR (targeting a non-overlapping region of the genome) and sequencing of the amplicon

Reference Laboratories should be contacted for specimen referral when testing laboratories cannot undertake any of the recommended test methods and testing is being undertaken that will result in notification to the OIE.

6.2 Clinically affected animals

No clinical signs are pathognomonic for infection with VHSV however, they may narrow the range of possible diagnoses.

6.2.1. Definition of suspect case in clinically affected animals

The presence of infection with VHSV shall be suspected if at least one of the following criteria is met:

i) Gross pathology or clinical signs associated with infection with VHSV as described in this chapter, with or without elevated mortality;

ii) Histopathological changes consistent with infection with VHSV as described in this chapter;

iii) A positive result from real-time PCR~~, conventional PCR, or IFAT;~~

iv) A positive result from a conventional PCR;

v) A positive result by IFAT

vi) VHSV-typical CPE ~~Cytopathic effect~~ in cell culture.

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with VHSV shall be confirmed if, in addition to the criteria in Section 6.2.1., ~~positive results has been obtained on at least one animal from two tests used in the following combination~~ one or more of the following criteria is met:

i) VHSV isolation in cell culture, followed by virus identification by real-time RT-PCR, Ag-ELISA, or IFAT and detection of VHSV in tissue preparations by conventional RT-PCR and sequencing of the amplicon;

ii) VHSV isolation in cell culture, followed by virus identification by real-time RT-PCR, Ag-ELISA, or IFAT and detection of VHSV in tissue preparations by real-time RT-PCR;

iii) Detection of VHSV in tissue preparations by real-time RT-PCR, and by a conventional RT-PCR (targeting a non-overlapping region of the genome) and sequencing of the amplicon.

~~i)~~ ~~VHSV isolation in cell culture, followed by virus identification by conventional RT-PCR, and sequencing of the amplicon;~~

~~ii)~~ ~~VHSV isolation in cell culture, followed by virus identification by real-time RT-PCR, Ag-ELISA, or IFAT and detection of VHSV in tissue preparations by conventional RT-PCR and sequencing of the amplicon;~~

~~iii) VHSV isolation in cell culture, followed by virus identification by real-time RT-PCR, Ag-ELISA, or IFAT and detection of VHSV in tissue preparations by real-time RT-PCR;~~

~~iv) VHSV isolation in cell culture, followed by virus identification by real-time RT-PCR, Ag-ELISA, or IFAT and a positive result from immunohistopathology);~~

~~v) Detection of VHSV in tissue preparations by real-time RT-PCR and by conventional RT-PCR, followed by sequencing of the amplicon.~~

Reference Laboratories should be contacted for specimen referral when testing laboratories cannot undertake any of the recommended test methods and testing is being undertaken that will result in notification to the OIE.

6.3. Diagnostic sensitivity and specificity for diagnostic tests

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with VHSV are provided in Table 6.3. This information can be used for the design of surveys for infection with VHSV, however, it should be noted that diagnostic performance is specific to the circumstances of each diagnostic accuracy study (including the test purpose, source population, tissue sample types and host species) and diagnostic performance may vary under different conditions. Data is only presented where tests are validated to atleast level two of the validation pathway described in Chapter 1.1.2. and the information is available within published diagnostic accuracy studies.

Table 6.1. *Diagnostic performance of tests recommended for surveillance or diagnosis*

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation
Cell culture	Surveillance	Experimentally infected fish	Kidney, heart and spleen	Rainbow trout	86 (84)	–	Real-time RT-PCR	Jonstrup *et al.*, 2013
Cell culture	Clinical diagnosis	Experimentally infected fish	Kidney	Atlantic salmon	100 (100)	94.4 (100)	Pseudo-gold standard*	Garver *et al.*, 2011
Real-time RT-PCR	Surveillance	Experimentally infected fish	Kidney	Atlantic salmon	93 (30)	100 (70)	Cell culture	Garver *et al.*, 2011
Real-time RT-PCR	Surveillance	Experimentally infected fish	Kidney, heart and spleen	Rainbow trout	90 (84)	100 (43)	Cell culture	Jonstrup *et al.*, 2013

* a compilation of 8 test results to evaluate both the RT-qPCR and virus isolation assay (Garver *et al*, 2011); DSe = diagnostic sensitivity, DSp = diagnostic specificity; *n* = number of samples used in the study .

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NB: There are OIE Reference Laboratories for Infection with viral haemorrhagic septicaemia virus (see Table at the end of this *Aquatic Manual* or consult the OIE web site for the most up-to-date list: http://www.oie.int/en/scientific-expertise/reference-laboratories/list-of-laboratories/).
Please contact the OIE Reference Laboratories for any further information on infection with viral haemorrhagic septicaemia virus

NB: FIRST ADOPTED IN 1995 AS VIRAL HAEMORRHAGIC SEPTICAEMIA;
MOST RECENT UPDATES ADOPTED IN 2012.

CHAPTER 2.3.3.

INFECTION WITH *GYRODACTYLUS SALARIS*

1. Scope

For the purpose of this chapter, *Gyrodactylus salaris* means infection with the pathogenic agent *G. salaris* (*G. salaris*) of the Genus *Gyrodactylus* and Family *Gyrodactylidae*, Order *Gyrodactylidea*, and Class *Monogenea*.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

Several strains or clades of *G. salaris* have been identified on the basis of genotyping with the mitochondrial cytochrome oxidase 1 (CO1) marker (Hansen *et al.,* 2003; 2007b; Meinila *et al.,* 2002; 2004; Mieszkowska *et al.,* 2018). Although there does not seem to be an unambiguous correspondence between parasite strains as identified by CO1 and pathogenicity (Hansen *et al*., 2007a), all strains recovered from Atlantic salmon (*Salmo salar*) that have been studied in laboratory experiments, so far, are highly pathogenic to strains of Atlantic salmon. Strains non-pathogenic to Atlantic salmon have been recovered from non-anadromous Arctic charr (*Salvelinus alpinus*) in Norway (Olstad *et al*., 2007a; Robertsen *et al*., 2007) and rainbow trout (*Oncorhynchus mykiss*) in Denmark (Jorgensen *et al*., 2007; Lindenstrom *et al*., 2003).

There has been a long taxonomic/scientific debate on whether *Gydrodacytlus. thymalli*, a species described from grayling (*Thymallus thymallus*), is a junior synonym of *G. salaris* (see e.g. Hansen *et al*., 2003; 2007a, 2007b; Meinila *et al*, 2004, Fromm *et al*, 2014), and most evidence favours such a synonymisation. The National Center for Biotechnology Information (NCBI) has accepted the synonymisation of *G. salaris* and *G. thymalli* with the result that all accessions of DNA sequences previously assigned to *G. thymalli* are now assigned to *G. salaris*. Irrespective of this debate, strains isolated from grayling have never been found pathogenic to Atlantic salmon in experimental trials (see e.g. Sterud *et al*., 2002), and do not seem to occur on Atlantic salmon when in sympatry with grayling (Anttila *et al*., 2008). In this chapter, it is assumed that *G. salaris* and *G. thymalli* are two species.

2.1.2. Survival and stability on host tissues

Survival of *G. salaris* attached to a dead host is temperature dependent: maximum survival times for *G. salaris* on dead Atlantic salmon are 72, 142 and 365 hours at 18, 12 and 3°C, respectively (Olstad *et al*., 2006).

2.1.3. Survival and stability off the host or in processed or stored samples

Survival of detached *G. salaris* is temperature dependent: approximately 24 hours at 19°C, 54 hours at 13°C, 96 hours at 7°C and 132 hours at 3°C (Olstad *et al*., 2006). *Gyrodactylus salaris* is known to survive between temperatures of 0°C to 25°C. Tolerance to temperatures above 25°C is unknown. *Gyrodactylus salaris* is sensitive to freezing and desiccation. It dies after a few days at pH≤5. It is more sensitive to low pH (5.1<pH<6.4) in association with aluminium and zinc than the host Atlantic salmon (Poleo *et al*., 2004; Soleng *et al*., 1999), and recently, it was also found that *G. salaris* is sensitive to low doses of chlorine (Hagen *et al*., 2014). For inactivation methods, see Section 2.4.5.

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with *G. salaris* according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) include: Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), Arctic char (*Salvelinus alpinus*), brown trout (*Salmo trutta*), grayling (*Thymallus thymallus*), and North American brook trout (*Salvelinus fontinalis*).

2.2.2. Species with incomplete evidence for susceptibility

None known.

2.2.3. Non-susceptible species

Species that have been found non-susceptible to infection with *G. salaris* according to Chapter 1.5. of the *Aquatic Code* are: [under study].

2.2.4. Likelihood of infection by species, host life stage, population or sub-populations

The prevalence and abundance of *G. salaris* on Atlantic salmon are higher than in other susceptible species. All life stages are susceptible, but prevalence and abundance in Atlantic salmon are highest in fry and parr stages, where mortality is also most likely to be observed.

2.2.5. Distribution of the pathogen on the host

Gyrodactylus salaris usually occurs on the fins of infected Atlantic salmon, but the parasite distribution on the host may vary depending on intensity of infection (Jensen & Johnsen, 1992; Mo, 1992; Paladini *et al*., 2014). Parasites are also commonly found on the body but less commonly on the gills. On other hosts, the distribution may be different, but in general the parasite is relatively less abundant on the fins and relatively more common on the body compared with Atlantic salmon.

2.2.6. Aquatic animal reservoirs of infection

There are a number of combinations of host species and *G. salaris* strains which do not result in clinical signs of disease and may, therefore, act as reservoirs of infection. Some stocks of Atlantic salmon in the Baltic region are infected with *G. salaris* but do not generally show clinical signs or suffer mortality (Anttila *et al.,* 2008). *Gyrodactylus salaris* has been found in wild Artic charr without any observable signs or mortality (Robertsen *et al.,* 2007). Rainbow trout can be infected with some strains of *G. salaris* at a very low prevalence and abundance without observable signs (Paladini *et al.,* 2014).

2.2.7. Vectors

Gyrodactylus salaris parasites may attach themselves to species not considered susceptible species, for short periods of time. Thus, any fish species could act as a vector, however, there is no evidence that they are important in the epidemiology of *G. salaris*.

2.3. Disease pattern

2.3.1. Mortality, morbidity and prevalence

Mortality in farmed Atlantic salmon fry and parr can be 100% if not treated. Mortality in wild Atlantic salmon fry and parr in Norwegian rivers can be as high as 98%, with an average of about 85% (Johnsen *et al*, 1999). Mortality in other susceptible species is usually low to negligible.

Prevalence in susceptible strains of Atlantic salmon reaches close to 100% in wild parr in rivers (Appleby & Mo, 1997); similarly, prevalence in farmed Atlantic salmon (in freshwater) rises to close to 100% within a short time after introduction of the parasite. Prevalence in resistant strains of Atlantic salmon in rivers and farms is unknown. Prevalence in other susceptible species is usually much lower than in Atlantic salmon and can be below 10% (e.g. in farmed rainbow trout; Buchmann & Bresciani, 1997).

2.3.2. Clinical signs, including behavioural changes

Usually there are no clinical signs in wild Atlantic salmon with infections of one or up to a few tens of *G. salaris* parasites. In the early disease phase in susceptible stocks of wild Atlantic salmon, increased flashing (fish scratch their skin on the substrate) is typical. Later, fish may become greyish because of increased mucous production and the fins may be eroded. Diseased fish are lethargic and are usually found in slower-moving water.

Flashing is common among moderate to heavily infected farmed Atlantic salmon as they scratch their skin on the bottom or wall of a tank or pond. Heavily infected fish may have reduced activity and stay in low current areas.

Rainbow trout usually only carry low numbers of *G. salaris* parasites and do not show clinical signs.

2.3.3 Gross pathology

Heavily infected Atlantic salmon may become greyish as a result of increased mucification, and at a later stage the dorsal and pectoral fins may become whitish as a result of increased thickness (mainly hypertrophy) of the epidermis. As the infestation continues, fish may have eroded fins, especially dorsal, tail and pectoral fins, because of parasite feeding. Secondary fungal infections (*Saprolegnia* spp.) are commonly observed in fish with infection with *G. salaris*.

2.3.4. Modes of transmission and life cycle

Gyrodactylus salaris is an obligate parasite with a direct life cycle. Parasites give birth to live offspring, and there are no other life stages. *Gyrodactylus salaris* can transfer to a new host via contact with live hosts, dead hosts, detached parasites drifting in the water column, or parasites attached to the substrate.

Gyrodactylus salaris has spread between rivers and farms mainly by the translocation of live fish. Fish migrating through brackish water can also spread the parasite between neighbouring rivers (see also Section 2.3.5). The risk of transmission is greater between rivers located within the same brackish water system.

2.3.5. Environmental and management factors

Although *G. salaris* mainly lives in fresh water, it reproduces normally at salinities up to 5–6 ppt. Survival at higher salinities is temperature dependent. For example at 1.4°C, *G. salaris* may survive for 240 hours, 78 hours and 42 hours at 10 ppt, 15 ppt and 20 ppt salinity, respectively, while at 12°C it may survive for 72 hours, 24 hours and 12 hours at the same three salinities, respectively (Soleng & Bakke, 1997).

Gyrodactylus salaris is sensitive to changes in the chemical composition of the water. It is sensitive to the most commonly used chemicals for bath treatment of farmed salmon parr and eggs (e.g. high salinity saltwater, formaldehyde and compounds containing chlorine and iodine). Furthermore, *G. salaris* is sensitive to acidic solutions (pH 5.0–6.0) of aluminium sulphate ([$Al_2(SO_4)_3$]) and zinc (ZN) (Poleo *et al*., 2004; Soleng *et al*., 1999). As aluminium sulphate is less toxic to fish than to *G. salaris* in moderately acidified waters, and this chemical has been used to eradicate the parasite from one river system in Norway (Pettersen *et al*., 2007). *Gyrodactylus salaris* is sensitive to low doses of chlorine (Hagen *et al*. 2014).

2.3.6. Geographical distribution

Gyrodactylus salaris is restricted in its distribution to Europe. It has been recovered from farmed Atlantic salmon or farmed rainbow trout in several (mainly northern) European countries. In the wild, the parasite has been found on wild salmonids, mainly Atlantic salmon parr, in rivers in Russia, Sweden, Finland and Norway. In some areas, the parasite continues to spread, and in 2015 it was detected on salmon parr in a new area in the north of Russia. In 2006, infection with *G. salaris* was reported from fish farms in Italy (Paladini *et al*., 2009) and, in 2007, from fish farms in Poland (Rokicka *et al*., 2007) and Macedonia (Zietara *et al*., 2007). In 2009, *G. salaris* was identified from fish farms in Romania (Hansen *et al*., 2014). The parasite has never been detected in the United Kingdom or in the Republic of Ireland.

For recent information on distribution at the country level consult the WAHIS interface (https://www.oie.int/wahis_2/public/wahid.php/Wahidhome/Home/index/newlang/en).

2.4. Biosecurity and disease control strategies

2.4.1. Vaccination

Vaccines are not available.

2.4.2. Chemotherapy including blocking agents

Not applicable.

2.4.3. Immunostimulation

Immunostimulation is not available.

2.4.4. Breeding resistant strains

In laboratory experiments, selected breeding of Atlantic salmon has resulted in increased survival among the offspring (Salte *et al*., 2010). However, stocking rivers with resistant strains has not been attempted because the stock will remain infected and thus the parasite may spread to other rivers with susceptible hosts.

2.4.5. Inactivation methods

Not applicable.

2.4.6. Disinfection of eggs and larvae

Eggs that are transferred from infected farms should be disinfected (iodine-containing compounds have been used).

2.4.7. General husbandry

Treatment of farmed salmonid populations with formaldehyde or other bath treatments will reduce the prevalence and abundance of *G. salaris* and may therefore render detection more difficult.

Restocking with resistant strains of Atlantic salmon (e.g. Baltic Neva strain) in affected rivers is not considered compatible with existing strain management of Atlantic salmon (i.e. preservation of the genetic integrity of wild stocks) (Karlsson *et al.*, 2019).

The spread of *G. salaris* between freshwater fish farms and between rivers may be avoided by disinfection of equipment (e.g. fish nets) before translocation.

3. Specimen selection, sample collection, transportation and handling

This section draws on information in Sections 2.2, 2.3 and 2.4 to identify populations, individuals and samples that are most likely to be infected.

3.1. Selection of populations and individual specimens

Sampling wild healthy populations should take place during the late summer or autumn when prevalence is highest. Atlantic salmon should be targeted. In farms, fish showing clinical signs of infection (as described in Section 2.3.1) should be selected. Sampling should be avoided for a period after treatment for ectoparasites. In the absence of clinical signs sampling in wild populations should target year class 1+ and 2+ as these are more likely of being infected than 0+ parr.

3.2. Selection of organs or tissues

Detection of *Gyrodactylus* and identification of *G. salaris* is a two-step process. Firstly, gyrodactylid parasite specimens are detected (e.g. on fish or fins) using optical equipment and picked out, and individual parasites are identified to species level using other equipment and methods.

Fish should be examined as whole specimens either live under anaesthesia (for example, with MS222), freshly killed, or preserved. In addition, fresh or preserved fins can be examined. Examination of live, anaesthetised fish is very time-consuming and not recommended. When Atlantic strains of Atlantic salmon parr are infected, almost all fish have at least one *G. salaris* on one of the fins. On some fish, *G. salaris* specimens may occur on the body or head, including the nostrils, the gills and the mouth cavity. The distribution of *G. salaris* on fins and other parts of the fish varies among fish species and strains of Atlantic salmon. For all hosts the examination of whole fish is recommended as it will increase the likelihood of detecting low intensity infections.

Live anaesthetised fish, freshly cut fins or EtOH-preserved fish or fins should be examined under a binocular dissecting microscope with good illumination. The fish should be placed in a box and completely covered in fresh water. Preserved fish can also be examined in EtOH. Living parasites are more easily detected by their movements, thus disturbing light refraction on the skin of the fish should be avoided. Live *Gyrodactylus* are colourless while EtOH-preserved *Gyrodactylus* specimens are usually slightly opaque. Dark field illumination microscopy will increase the contrast and the parasites will be detected more easily. The whole surface of the fish, including gills and mouth cavity, must be examined. It is best to use two forceps for this process. The fins of relatively small fish, usually less than 10 cm, can also be studied using illumination through the bottom of the microscope stage, which makes *Gyrodactylus* specimens easy to observe.

3.3. Samples or tissues not suitable for pathogen detection

Dead fish, stored on ice, are not acceptable for *Gyrodactylus* examination, even if the fish are kept separately in plastic bags, etc. The parasites die quickly if not covered in water and rapidly disintegrate.

3.4. Non-lethal sampling

Fish can be examined as live specimens under anaesthesia (for example, with MS222). Recently, a non-lethal method for isolating specimens of gyrodactylid parasites from fish was developed and tested on brown trout (Thrush *et al*., 2019). The method was shown to have a higher parasite recovery rate compared to whole body examination of killed fish (84.6% and 51.9%, respectively). The method has not yet been used on fish infected with *G. salaris* but it is likely to be effective.

In addition, environmental DNA (eDNA) methods for detection of *G. salaris* and its two main hosts, Atlantic salmon and rainbow trout, in water samples have been developed (Rusch *et al*., 2018). However, detection limits have not been established for these analyses.

3.5. Preservation of samples for submission

Fish should be killed immediately and should not be allowed to dry out before preservation. Whole fish should be preserved in 80–100% EtOH in bottles large enough to provide excess space and preservative. The concentration of EtOH after preservation should not be below 70%. As a rule of thumb this concentration is obtained if the proportion of fish tissue to EtOH does not exceed 1:9. If the concentration is lower, the mucous and epidermis may disintegrate and *Gyrodactylus* specimens, even if they are preserved, may drop off. Bottles should have an opening wide enough to avoid the possibility of scraping off *Gyrodactylus* specimens when fish are put into the bottle or when taken out for examination. Bottles should be stored in a horizontal position until the tissue is fixed/preserved to prevent the fish curling. When preservation of the fish is complete, the bottles can be stored in a vertical position.

As *G. salaris* is common on fins of Atlantic salmon, fins cut off from the body and stored in EtOH as described above can also be submitted. This is especially suitable for larger fish and under field conditions where, for example, transport is limited.

Formaldehyde-fixed *Gyrodactylus* specimens are difficult to identify morphologically and are unsuitable for DNA analysis.

3.5.1. Samples for pathogen isolation

Not applicable.

3.5.2. Preservation of samples for molecular detection

Tissue samples, i.e. isolated parasites, whole fish or fins, for PCR testing should be preserved in 70–90% (v/v) analytical/reagent-grade (absolute) ethanol. The recommended ratio of ethanol to tissue is 9:1 based on studies in terrestrial animal and human health. The use of lower grade (laboratory or industrial grade) ethanol is not recommended.

Template DNA should be prepared from live/fresh or EtOH-preserved specimens using a suitable DNA preparation protocol. DNA extraction kits may be used according to the manufacturers' instructions.

3.5.3. Fixed samples for histopathology, immunohistochemistry or in-situ hybridisation

Not applicable.

3.5.4. Fixed samples for electron microscopy

Not applicable.

3.5.5. Samples for other tests

Preservation of samples for environmental DNA (*eDNA*) *analyses*

Several methods for filtering water for eDNA analyses exist and the method has also been developed for use on *G. salaris* and its hosts, *Salmo salar* and *Onchorhynchus mykiss* (Rusch *et al.*, 2018). Duplicate water samples of 5 litres (2 × 5 litres) should be collected and filtered on site on to glass fibre filters (47 mm AP25 Millipore, 2 µm pore size, Millipore, Billerica, USA) using a suitable pump, tubing and filter holder. Filters should be placed in separate zip-lock plastic bags containing silica gel and stored dry and dark until further analysis in the laboratory.

3.6. Pooling of samples

Sampled fish can be pooled, although each fish should subsequently be examined and analysed separately. Fins of fish from a farm or a river can be pooled and are also examined and analysed separately, but in this instance each fin cannot be related to a certain fish host. Material from parasites should not be pooled for molecular diagnostics.

4. Diagnostic methods

The methods currently available for identifying infection for surveillance (in healthy populations), presumptive and confirmatory diagnostic purposes are listed in Table 4.1. by life stage. The designations used in the Table indicate:

Key:

+++ =	Recommended method(s) validated for the purpose shown and usually to stage 3 of the OIE Validation Pathway; OIE recommended method(s) will be mentioned in the text;
++ =	Suitable method(s) but may need further validation;
+ =	May be used in some situations, but cost, reliability, lack of validation or other factors severely limits its application;
Shaded boxes =	Not appropriate for this purpose.

The selection of a test for a given purpose depends on sensitivity, specificity, repeatability and reproducibility. OIE Reference Laboratories welcome feedback on diagnostic performance for assays, in particular PCR methods, for factors affecting assay sensitivity or specificity, such as tissue components inhibiting amplification, nonspecific or uncertain bands, etc., and any assays that are in the +++ category.

Table 4.1. OIE recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals

Method	A. Surveillance of apparently healthy animals				B. Presumptive diagnosis of clinically affected animals				C. Confirmatory diagnosis1 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
Morphological examination		+	+	1		+	+	1				
Histopathology[3]												
Cytopathology[3]												
Culture												
Real-time PCR (using parasite sample)		+	+	1		+	+	1				
ddPCR/Real-time PCR (using environmental sample)	+			1								
Conventional PCR										++	++	2
Amplicon sequencing[4]										++	++	2
***In-situ* hybridisation**												
Bioassay												
LAMP												
Ab-ELISA												
Ag-ELISA												

LV = level of validation, refers to the stage of validation in the OIE Pathway (chapter 1.1.2); PCR = polymerase chain reaction; ddPCR = droplet digital PCR; LAMP = loop-mediated isothermal amplification; Ab- or Ag-ELISA = antibody or antigen enzyme-linked immunosorbent assay, respectively; [1]For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). [2]Early and juvenile life stages have been defined in Section 2.2.3. [3]Histopathology and cytopathology can be validated if the results from different operators has been statistically compared. [4]Sequencing of the PCR product. Shading indicates the test is inappropriate or should not be used for this purpose

4.1. Morphological examination

Morphological identification of *Gyrodactylus* species is based on the morphology and morphometry of marginal hooks anchors (hamuli) and bars in the opisthaptor (the attachment organ). Good preparation of specimens is a prerequisite for species identification. Morphological identification is only recommended for preliminary diagnosis of *G. salaris* and should not be used for confirmation, for which molecular methods are recommended.

Digestion of the soft tissue, leaving the hard parts only, is recommended when high-resolution morphometrics is required for reliable morphometric diagnosis. The soft tissue can be digested in a solution (approx. 1 µl) of 75 mM Tris, 10 mM EDTA (ethylene diamine tetra-acetic acid), 5% SDS (sodium dodecyl sulphate) and 100 mg ml^{-1} proteinase K, pH 8.0. After adding the digestion solution, the reaction should be inspected in the microscope until completion and then ended by adding a stop solution (1:1 glycerol and 10% neutral buffered formalin). The procedure for digestion is described in detail in Harris *et al*., 1999. Identification of *G. salaris* should be in accordance with references: Cunningham *et al*., 2001; Malmberg, 1957; 1970; McHugh *et al*., 2000; Olstad *et al*., 2007b; Shinn *et al*., 2004.

The size of the opisthaptoral hard parts in *Gyrodactylus* varies extensively with, for example, temperature, whereas shape is more stable (see e.g. Mo, 1991a). The capability of linear measurements to capture morphology might therefore not always be sufficient for reliable diagnosis (Olstad *et al*., 2007b).

Gyrodactylus salaris can be differentiated from *Gyrodactylus* species by trained morphologists on the basis of morphology but not from *G. thymalli* (Olstad *et al*., 2007b). In addition, *G. salaris* is morphologically similar to *Gyrodactylus teuchis* from brown trout, Atlantic salmon, and rainbow trout, but can be differentiated by trained morphologists on the basis of the shape of the marginal hook sickle. *Gyrodactylus teuchis* has a longer and more constantly curved sickle blade (see Cunningham *et al*., 2001).

4.2. Histopathology and cytopathology

Not applicable.

4.3. Cell or artificial media culture for isolation

Not applicable.

4.4. Nucleic acid amplification

For all molecular tests below DNA can be extracted using standard DNA extraction kits.

4.4.1. Real-time PCR

Both real-time PCR (Collins *et al*., 2010) and digital droplet (dd) PCR (Rusch *et al*., 2018) have been developed for *G. salaris*. Real-time PCR has not been widely applied for diagnostics of *G. salaris,* and ddPCR is developed for use in connection with eDNA-methods. Both these methods target the ribosomal internal transcribed spacers region (ITS) and have the same diagnostic limitations as described in Sections 4.5.1 and 4.5.2. However, real-time PCR is faster than conventional PCR and DNA sequencing (Section 4.4.2) and can be applied as a fast mean to exclude other species than *G. salaris/G. thymalli*, and the method is therefore mentioned briefly here. Conventional PCR and sequencing of the mitochondrial cytochrome oxidase I gene (Sections 4.4.2 and 4.5.2), which is necessary for species confirmation and haplotype identification, can then be performed on those species with a positive result from real-time PCR.

The real-time PCR assay of Collins *et al.* (2010) is a TaqMan minor groove binder (MGB) real-time PCR assay that targets a 60 bp unique sequence motif in the ITS1 region of *G. salaris*/*G.thymalli*. It applies the forward primer F (5'-CGA-TCG-TCA-CTC-GGA-ATC-G-3'), reverse primer R (5'-GGT-GGC-GCA-CCT-ATT-CTA-CA-3') and TaqMan MGB probe Gsal2 (5'-FAM-TCT-TAT-TAA-CCA-GTT-CTG-C-3') labelled with the fluorescent reporter dye FAM at the 5'-end and a non-fluorescent quencher MGBNFQ at the 3'-end. Amplifications were performed in a total volume of 20 µl containing TaqMan Universal PCR Master mix (with UNG; Applied Biosystems), 0.9 µM of each forward and reverse primer and 0.25 µM of each probe and dH2O (Sigma) to a final volume of 20 µl. One µl of lysate from a parasite specimen was added to the each test tube. The cycling conditions were 50°C for 2 min, 95°C for 10 min followed by 35 cycles of 95°C for 15 s and 60°C for 1 min and run in a ABI 7000 Sequence Detection System (Applied Biosystems). The efficiency of the singleplex assay were reported as ranging from 93.1 to 101.1% and the limit of detection (dilution) as 10^{-4}. Further details can be found in Collins *et al.* (2010). **Note:** A low level of cross-amplification of *Gyrodactylus derjavinoides* has been observed using the real-time PCR set-up described here (Rusch *et al.,* 2018).

4.4.2. Conventional PCR

Analysis of the ribosomal RNA gene internal transcribed spacer region (*ITS)*

For amplification of a 1300 base pair product of the ITS-region, covering ITS1, 5.8S, and ITS2, primers, such as 5'-TTT-CCG-TAG-GTG-AAC-CT-3' and 5'-TCC-TCC-GCT-TAG-TGA-TA-3', may be used. The cycling conditions for PCR are as follows, initial denaturation at 95°C for 5 minutes; 30 cycles of 94°C for 1 minute, 50°C for 1 minute, 72°C for 2 minutes; final extension at 72°C for 7 minutes (Cunningham, 1997). If partially degraded material is analysed or if the PCR above does not give a positive result, the ITS1 and ITS2 spacers can be amplified in two separate reactions using primer sets and PCR conditions described in Matejusová *et al.*, 2001. The amplification of ITS2 alone, using the primers 5'-CAT-CGG-TCT-CTC-GAA-CG-3' and 5'-TCC-TCC-GCT-TAG-TGA-TA-3' and using the same protocol as above is sufficient.

The primers for amplification of ITS are not specific to *G. salaris* and will amplify all or most species of *Gyrodactylus*. Positive PCR products should thus be sequenced for species confirmation (Section 4.5).

Analysis of the mitochondrial cytochrome oxidase I (*CO1*) *gene*

For amplification of the CO1-gene, the primers 5'-ATA-TAG-ACG-ATT-TGT-TTT-CA-3' and 5'-ACA-GAT-TAC-TTG-GTA-TTA-CA-3' (Kuusela *et al.,* 2009) may be used to amplify the full-length gene (1600 base pairs) which is recommended. The primers 5'-TAA-TCG-GCG-GGT-TCG-GTA-A-3' and 5'-GAA-CCA-TGT-ATC-GTG-TAG-CA-3') (Meinila *et al.,* 2002) may be used to amplify a 800 base pairs fragment if the first PCR is unsuccessful. The cycling conditions for both PCRs are as follows, initial denaturation at 95°C for 5 minutes; 35 cycles of 95°C for 1 minute, 50°C for 1 minute, 72°C for 2 minutes; final extension at 72°C for 7 minutes. Additional primer sets for amplification of CO1 can be found in references: Meinila *et al.,* 2002; 2004, Kuusela *et al.,* 2009.

Primers recommended for amplification of CO1 might not be specific for *G. salaris*. Positive PCR products should thus be sequenced for species confirmation (Section 4.5).

The following controls should be run with each assay: negative extraction control; positive control; no template control.

4.4.3. Other nucleic acid amplification methods

Not applicable.

4.5. Amplicon sequencing

4.5.1. ITS sequencing and sequence analysis

Amplified ITS fragments prepared as in Section 4.4.2 should be sequenced using the PCR primers and, in addition, internal sequencing primers (Cunningham, 1997; Matejusová *et al.*, 2001) should be used to obtain overlapping reads of each nucleotide. The resulting ITS sequences should be subjected to a BLAST search in GenBank/EMBL to establish identity with known sequences. Several sequences of other species infecting salmonids, e.g. *G. derjavini*, *G. derjavinoides*, *G. truttae*, and *G. teuchis* are available in GenBank/EMBL. *G. thymalli* cannot be distinguished from *G. salaris* by this method, but sequences of ITS distinguishes *G. salaris* from all other known species. If the BLAST search identifies the parasite as *G. salaris*, CO1 sequencing and sequence analysis should be performed (Section 4.5.2).

4.5.2. CO1 sequencing and sequence analysis

Amplified CO1 fragments prepared as described in Section 4.4.2 should be sequenced and, in addition, internal sequencing primers (Kuusela *et al*., 2009; Meinila *et al*., 2002) should be used to obtain overlapping reads of each nucleotide. The resulting CO1 sequences should be subjected to a BLAST search in GenBank/EMBL to identify the haplotype.

If the obtained sequence does not have a 100% match in GenBank/EMBL, a phylogenetic analysis can be performed to establish the relationship to other available sequences. Different haplotypes and clades of *G. salaris* and *G. thymalli* can be distinguished with this method. CO1 sequences can be used to assign specimens to a haplotype or clade and thus infer the identity as *G. salaris* or *G. thymalli*. Clades (haplogroups) of *G. salaris* generally correspond well to host preferences and/or the geographical distribution of the parasites, with a few exceptions, and some strains, as defined by CO1-sequences (haplotypes), are known to be pathogenic to Atlantic salmon. Host identity can be used to infer potential pathogenicity of a certain strain and thus host identity of sequence hits in GenBank/EMBL should always be checked when BLAST results are returned.

Where the sequence is not assigned to one of the recognised haplotypes (CO1 sequences) of *G. salaris* or *G. thymalli* advice should be sought from the OIE Reference Laboratory. The OIE Reference laboratory will keep an updated database of CO1-sequences and will assist in the diagnosis. It is recommended that the OIE Reference laboratory is informed of any significant detections of *G. salaris* and *G. thymalli* in order to confirm the cases.

4.6. In-situ hybridisation

Not applicable.

4.7. Immunohistochemistry

Not applicable.

4.8. Bioassay

Not applicable.

4.9. Antibody- or antigen-based detection methods (ELISA, etc.)

Not applicable.

4.10. Other methods

Not applicable.

5. Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations

Real-time PCR is the recommended test for surveillance to demonstrate freedom of disease in apparently healthy population. Sequencing of the amplified CO1 amplicon is required for confirmation of infection in any parasite that identified as positive PCR

6. Corroborative diagnostic criteria

All suspect positive samples of *G. salaris* from country or zone or compartment considered free from infection with *G. salaris* should be referred immediately to the OIE Reference Laboratory for confirmation, whether or not clinical signs are associated with the case.

This section only addresses the diagnostic test results for detection of infection in the absence (Section 6.1) or presence of clinical signs (Section 6.2) but does not evaluate whether the infectious agent is the cause of the clinical event.

The case definitions for a suspect and confirmed case have been developed to support decision making related to trade and confirmation of disease status at the country, zone or compartment level. Case definitions for disease confirmation in endemically affected areas may be less stringent.

6.1. Apparently healthy animals or animals of unknown health status[5]

Healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link(s) to an infected population. Geographic proximity to, or movement of animals or animal products or equipment, etc., from a known infected population equate to an epidemiological link. Alternatively, healthy populations will be sampled in surveys to demonstrate disease freedom.

6.1.1. Definition of suspect case in apparently healthy animals

The presence of infection with *G. salaris* shall be suspected if at least one of the following criteria is met:

i) Identification of *G. salaris* by morphological examination

II) A positive result by real-time PCR

iii) A positive result by ddPCR or real-time-PCR from an environmental sample.

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with *G. salaris* is considered to be confirmed if, in addition to the criteria in Section 6.1.1., the following criterion is met:

i) A positive result from sequencing amplified CO1 fragments obtained by conventional PCR.

6.2 Clinically affected animals

Clinical signs are not pathognomonic for a single disease; however, they may narrow the range of possible diagnoses.

6.2.1. Definition of suspect case in clinically affected animals

The presence of infection with *G. salaris* shall be suspected if at least one of the following criteria is met:

i) Gross pathology or clinical signs associated with the disease as described in this chapter, with or without elevated mortality

ii) Identification of *G. salaris* by morphological examination

iii) A positive result by real-time PCR.

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with *G. salaris* is considered to be confirmed if, in addition to the criteria in section 6.2.1. the following criterion is met:

i) A positive result from sequencing of amplified CO1 fragments obtained by conventional PCR.

6.3. Diagnostic sensitivity and specificity for diagnostic tests

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with *G. salaris* are provided in Table 6.3. (note: no data are currently available). This information can be used for the design of surveys for infection with *G. salaris*, however, it should be noted that diagnostic performance is specific to the circumstances of each diagnostic accuracy study (including the test purpose, source population, tissue sample types and host species) and diagnostic performance may vary under different conditions. Data is only presented where tests are validated to at least level two of the validation pathway described in Chapter 1.1.2. and the information is available within published diagnostic accuracy studies.

[5] For example, transboundary commodities.

Table 6.3. *Diagnostic performance of tests recommended for surveillance or diagnosis*

Test type	Test purpose	Source population	Tissue/ sample type	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation
Real-time PCR	Surveillance	–	Parasites	–	Not yet available	Not yet available	–	–
Amplicon sequencing	Diagnosis	–	Parasites	–	Not yet available	Not yet available	–	–

DSe = diagnostic sensitivity; DSp = diagnostic specificity; *n* = number of samples used in the study;

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* *

NB: There is an OIE Reference Laboratory for infection with *G. salaris*
(see Table at the end of this *Aquatic Manual* or consult the OIE web site for the most up-to-date list:
http://www.oie.int/en/scientific-expertise/reference-laboratories/list-of-laboratories/).
Please contact the OIE Reference Laboratories for any further information on infection with *G. salaris*.

NB: FIRST ADOPTED IN 1997 AS GYRODACTYLOSIS OF ATLANTIC SALMON (*GYRODACTYLUS* F);
MOST RECENT UPDATES ADOPTED IN 2018.

CHAPTER 2.3.6.

INFECTION WITH SALMONID ALPHAVIRUS

1. Scope

Infection with salmonid alphavirus (SAV) means infection with any genotype of the pathogenic agent SAV, Genus *Alphavirus* and Family *Togaviridae.*

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

SAV is an enveloped, spherical, single-stranded, positive-sense RNA virus, approximately 60–70 nm in diameter, with a genome of ~12 kb. The genome codes for eight proteins: four capsid glycoproteins (E1, E2, E3 and 6K) and four nonstructural proteins (nsP1–4). Glycoprotein E2 is considered to be the site of most neutralising epitopes, while E1 contains more conserved, cross-reactive epitopes (McLoughlin & Graham, 2007). SAV is considered to belong to the Genus *Alphavirus* of the Family *Togaviridae*. This is based on nucleotide sequence studies of SAV isolates, and is also supported by biological properties of the virus, including cross-infection and neutralisation trials. In addition, four conserved nucleotide sequence elements (CSEs) and a conserved motif (GDD), characteristic of alphaviruses, are present in the SAV genome (McLoughlin & Graham, 2007).

SAV has been divided into six genotypes (SAV 1–SAV 6) based solely on nucleic acid sequences for the proteins E2 and nsP3 (Fringuelli *et al.,* 2008). The level of antigenic variation among genotypes is considered low as monoclonal antibodies (MAbs) raised against a specific SAV genotype are likely to cross react with other SAV isolates (Graham *et al.,* 2014; Jewhurst *et al.,* 2004). The genotype groups by susceptible species and environment are presented in Table 2.1.

Infection with SAV causes pancreas disease (PD) or sleeping disease (SD) in Atlantic salmon (*Salmo salar* L.), common dab (*Limanda limanda*), rainbow trout (*Oncorhynchus mykiss*) (McLoughlin & Graham, 2007) and Arctic charr (*Salvelinus alpinus*) (Lewisch *et al*., 2018). The disease is systemic, characterised microscopically by necrosis and loss of exocrine pancreatic tissue, and heart and skeletal muscle necrosis and atrophy.

Table 2.1. *SAV genotypes by susceptible host species and environment*

SAV genotype	Freshwater	Sea water
SAV 1	Rainbow trout	Atlantic salmon
SAV 2	Rainbow trout; Atlantic salmon; Arctic charr	Atlantic salmon
SAV 3		Rainbow trout; Atlantic salmon
SAV 4		Atlantic salmon
SAV 5		Atlantic salmon; Common dab
SAV 6		Atlantic salmon

2.1.2. Survival and stability in processed or stored samples

There are no published scientific data specifically on the survival and stability of SAV in processed or stored samples. The OIE Reference Laboratory has found that SAV in serum/plasma samples and virus isolated from cell culture can be stored for many years at –80°C without significant decline in virus titre. This observation is consistent with research on other alphaviruses.

2.1.3. Survival and stability outside the host

Laboratory tests suggest that SAV would survive for extended periods in the aquatic environment. In these tests, virus survival was inversely related to temperature. In the presence of organic matter, markedly longer survival times were observed in sea water compared with fresh water (Graham *et al.,* 2007b).

The half-life of SAV in serum has been found to be inversely related to temperature, emphasising the need for rapid shipment of samples at 4°C to laboratories for virus isolation. For long-term conservation of SAV-positive samples and cultured virus, storage at –80°C is recommended (Graham *et al.,* 2007b).

For inactivation methods, see Section 2.4.5.

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with SAV according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) include: Arctic charr (*Salvelinus alpinus*), Atlantic salmon (*Salmo salar*), common dab (*Limanda limanda*) and rainbow trout (*Oncorhynchus mykiss*).

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence for susceptibility according to Chapter 1.5. of the *Aquatic Code* include: long rough dab (*Hippoglossoides platessoides*), plaice (*Pleuronectes platessa*) and Ballan wrasse (*Labrus bergylta*).

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following species, but an active infection has not been demonstrated: Argentine hake (*Merluccius hubbsi*), brown trout (*Salmo trutta*), cod (*Gadus morhua*), European flounder (*Platichthys flesus*), haddock (*Melanogrammus aeglefinus*), herring (*Clupea harengus*), Norway pout (*Trisopterus esmarkii)*, saithe (*Pollachius virens*), longhorn sculpin (*Myoxocephalus octodecemspinosus*) and whiting (*Merlangius merlangus*).

2.2.3. Non-susceptible species

Species that have been found non-susceptible to infection with SVCV according to Chapter 1.5. of the *Aquatic Code* are: No species are listed as non-susceptible.

2.2.4. Likelihood of infection by species, host life stage, population or sub-populations

Farmed Atlantic salmon and rainbow trout are the species with the highest likelihood of infection with SAV. Experimental studies have demonstrated that all life stages are susceptible to infection (Taksdal & Sindre, 2016). SAV 1–SAV 6 have been detected in Atlantic salmon. SAV 2 and SAV 3 have been detected in rainbow trout.

2.2.5. Distribution of the pathogen in the host

The heart and the pancreas are main target organs for infection with SAV. Necrosis and loss of exocrine pancreatic tissue, myocarditis and skeletal myositis are typical histopathological findings. During the viraemic stage, substantial amounts of virus are also found in serum, and during the infection virus can also be found in kidney, spleen, gills, mucous and faeces (Taksdal & Sindre, 2016).

2.2.6. Aquatic animal reservoirs of infection

There is evidence that some survivors of outbreaks will become long-term carriers of the virus (Graham *et al*., 2009) and thus farmed Atlantic salmon and rainbow trout can be considered the main reservoir of SAV (Taksdal & Sindre, 2016). Infection with SAV has been detected in some wild flatfish species in Scotland (Bruno *et al.,* 2014; Snow *et al.,* 2010) which could also act as a reservoir of infection.

2.2.7. Vectors

Although most alphaviruses are transmitted by arthropod vectors, vector transmission of SAV has not yet been demonstrated. SAV has been detected by reverse-transcription (RT) PCR in salmon lice (*Lepeophtheirus salmonis*) collected during acute outbreaks of pancreas disease in Atlantic salmon, but transfer to susceptible fish species has not been reported (Petterson *et al.,* 2009).

2.3. Disease pattern

2.3.1. Mortality, morbidity and prevalence

Mortality rates due to infection with SAV may vary with genotype, season, year, use of biosecurity measures and species of fish (Bang Jensen *et al.,* 2012; Graham *et al.,* 2011; Rodger & Mitchell, 2007; Stormoen *et al*., 2013). The cumulative mortality at the farm level ranges from negligible to over 50% in severe cases (Bang Jensen *et al.,* 2012; Graham *et al.,* 2003; Rodger & Mitchell, 2007; Ruane *et al.,* 2008; Stene *et al.,* 2014). Experimental studies have demonstrated that SAV 2 infection in marine fish causes lower mortality than SAV 3 (Taksdal *et al.*, 2015).

Duration of disease outbreaks, defined as the period with increased mortality, may vary from 1 to 32 weeks (Jansen *et al.* 2010a; 2014; Ruane *et al.,* 2008).

The prevalence of infection with SAV may vary. During disease outbreaks, the prevalence is usually high; prevalences of 70–100% have been reported in Atlantic salmon farming sites (Graham *et al.,* 2010).

Prevalences in wild fish are largely unknown. SAV has been detected by PCR in some marine flatfish species in Scottish waters (Snow *et al.,* 2010). A serological survey of wild salmonids in freshwater river systems in Northern Ireland did not detect virus neutralisation antibodies against SAV in any of 188 sera tested, whereas the majority of sera from farmed salmon in sea water in the same area tested positive (Graham *et al.,* 2003).

2.3.2. Clinical signs, including behavioural changes

A sudden drop in appetite may be observed 1–2 weeks before the detection of elevated mortality. Clinically diseased fish may be observed swimming slowly at the water surface. In some cases, extremely weak (“sleeping”) fish can be found at the bottom of tanks or in net-cages. An increased number of faecal casts may also be observed. However, it is important to note that clinical signs are not pathognomonic.

Initially, nutritional status is usually normal, but in the months after an outbreak or in the later stages of disease, long slender fish (‘runts’) with poor body condition are typically observed. However, the presentation of long, slender fish can be caused by factors other than SAV.

2.3.3 Gross pathology

Yellow mucoid gut contents is a usual post-mortem finding, typically seen in inappettant fish. Occasionally, signs of circulatory disturbances, such as petechial haemorrhages, small ascites or reddening of the pancreatic region between the pyloric caeca, may be seen. Some diseased fish may show pale hearts or heart ruptures. It is important to note that post-mortem findings are not pathognomonic.

2.3.4. Modes of transmission and life cycle

Horizontal transmission of SAV is demonstrated by a range of evidence including: phylogenetic studies, successful transmission among cohabiting fish, proven transmission between farming sites, studies on survival of SAV in sea water and the spread via water currents (Graham *et al.,* 2007b; 2011; Jansen *et al.,* 2010a; Kristoffersen *et al.,* 2009; Viljugrein *et al.,* 2009).

Long-distance transmission, and thus introduction of SAV into a previously uninfected area, is most likely due to movement of infected live fish (Kristoffersen *et al.,* 2009; Rodger & Mitchell, 2007). SAV has been detected in fat leaking from dead fish which accumulates at the sea water surface, contributing to long distance spread of the virus (Stene *et al.,* 2013). Once SAV has been introduced into an area, farm proximity and water currents influence local transmission (Aldrin *et al.,* 2010; Kristoffersen *et al.,* 2009; Viljugrein *et al.,* 2009).

Vertical transmission of SAV has been suggested (Bratland & Nylund, 2009), but not demonstrated (Kongtorp *et al.,* 2010; McLoughlin & Graham, 2007). The Norwegian Scientific Committee for Food Safety, (2010), carried out a risk assessment and concluded that the risk of vertical transmission of SAV is negligible.

2.3.5. Environmental and management factors

Clinical outbreaks and mortality are influenced by water temperature and season (McLoughlin & Graham, 2007; Rodger & Mitchell, 2007; Stene *et al.,* 2014; Stormoen *et al.,* 2013). Stressing the fish by movement, crowding or treatment may initiate disease outbreaks on infected farms.

Risk factors for outbreaks on a farming site include a previous history of infection with SAV, high feeding rate, high sea lice burden, the use of autumn smolts and previous outbreaks of infectious pancreatic necrosis (IPN) (Bang Jensen *et al.,* 2012; Kristoffersen *et al.,* 2009; Rodger & Mitchell, 2007).

2.3.6. Geographical distribution

Infection with SAV has been reported from several countries in Europe. See WAHIS (https://www.oie.int/wahis_2/public/wahid.php/Wahidhome/Home/index/newlang/en) for recent information on distribution at the country level.

2.4. Biosecurity and disease control strategies

2.4.1. Vaccination

DNA-based and virus-inactivated vaccines against SAV are both commercially available. The vaccines may cause a risk of false positives, both in serological and PCR-based tests, according to data presented by vaccine companies. However, reports from the field indicates that false positives to serological tests do not occur after sea transfer. To prevent false positives by RT-PCR, sampling from vaccinated individuals should use heart tissue to avoid opening the abdominal cavity.

2.4.2. Chemotherapy including blocking agents

No chemotherapy is available.

2.4.3. Immunostimulation

No immunostimulation is available.

2.4.4. Breeding resistant strains

Differences in susceptibility among different family groups of Atlantic salmon have been observed in challenge experiments and in the field, indicating the potential for breeding for resistance (Norris *et al*., 2008; Gonen *et al.*, 2015). Breeding programmes in Ireland and Norway have successfully produced fish with increased resistance to disease caused by SAV, which are now commercially available.

2.4.5. Inactivation methods

SAV is rapidly inactivated in the presence of high levels of organic matter at 60°C, pH 7.2, and at 4°C, pH 4 and pH 12, suggesting that composting, ensiling and alkaline hydrolysis would all be effective at inactivating virus in fish waste (Graham *et al.,* 2007a). The virus is also readily inactivated by UV-light, but is more resistant to chlorine and ozone treatment.

2.4.6. Disinfection of eggs and larvae

Standard disinfection procedures are considered sufficient to prevent surface contamination of eggs by SAV (Graham *et al.,* 2007).

2.4.7. General husbandry

To avoid infection with SAV, good husbandry practices should be applied such as use of appropriate sites for farming, segregation of generations, stocking with good quality fish, removal of dead fish, regular cleaning of tanks and pens, control of parasites and other pathogens, as well as careful handling of fish. Once an outbreak has started, mortality may be reduced by minimising handling and ceasing feeding.

3. Specimen selection, sample collection, transportation and handling

3.1. Selection of populations and individual specimens

Clinical inspections should be carried out during a period when the water temperature is below XX°C. All production units (ponds, tanks, net-cages, etc.) should be inspected for the presence of dead, weak or abnormally behaving fish. Extremely weak ('sleeping') fish may be found at the bottom of a tank or in the net-cages. If the number of clinically diseased fish is low, samples from long, thin fish ('runts') may be added (Jansen *et al.,* 2010b). If moribund or thin fish or runts are sampled, the probability of detecting SAV is higher than if randomly selected, apparently healthy fish are sampled (Jansen *et al.,* 2010b). Prevalence estimates will also vary with the diagnostic method used.

Fish to be sampled are selected as follows:

i) Susceptible species should be sampled proportionally, or following risk-based criteria for targeted selection of lots or populations with a history of abnormal mortality or potential exposure events (e.g. via untreated surface water, wild harvest or replacement with stocks of unknown disease status).

ii) If more than one water source is used for fish production, fish from all water sources should be included in the sample.

iii) If weak, abnormally behaving or freshly dead (not decomposed) fish are present, such fish should be selected. If such fish are not present, the fish selected should include normal appearing, healthy fish collected in such a way that all parts of the farm as well as all year classes are proportionally represented in the sample.

3.2. Selection of organs or tissues

Heart and mid-kidney are the recommended organs for detection of SAV either by molecular biological methods or by cell culture. During the course of the disease, the heart usually contains more SAV than other tissues and should always be sampled. After disease outbreaks, gill and heart tissue (Graham *et al.,* 2010) and pools of heart and mid-kidney tissue (Jansen *et al.,* 2010b) remained positive by real time RT-PCR for months after initial detection.

For sampling from vaccinated fish, the heart should be sampled without opening the abdominal cavity. Sampling of mid-kidney, spleen or other internal organs is not recommended to avoid contamination of viral RNA/DNA from the vaccine (See Section 2.4).

During the initial viraemic phase, serum samples are also suitable for detection of SAV either by molecular biological methods or by cell culture, which can provide an early warning of disease outbreaks (Graham *et al.,* 2010). From approximately 3 weeks after SAV infection, blood serum or plasma is suitable for a virus neutralisation test (Graham *et al.,* 2003).

Tissues for histological examinations should include gill, heart, pyloric caeca with attached pancreatic tissue, liver, kidney, spleen and skeletal muscle containing both red (aerobic) and white (anaerobic) muscle. Skin with associated skeletal muscle should be sampled at the lateral line level and deep enough to include both red and white muscle.

3.3. Samples or tissues not suitable for pathogen detection

Pancreas, although a target organ for the virus, is not suitable for RT-PCR detection of SAV, as it is impossible to separate this organ from the intestine of the fish during sampling, and in addition loss of pancreas is common in infected fish. Organs other than those recommended in Section 3.2. should not be used for the detection of SAV, as the sensitivity of the diagnostic methods might be reduced.

3.4. Non-lethal sampling

There are investigations into using non-lethal sampling methods for surveillance of SAV in fish farms, including detection of virus in water. However, no validated methods are currently available.

3.5. Preservation of samples for submission

For guidance on sample preservation methods for the intended test methods, see Chapter 2.3.0.

3.5.1. Samples for pathogen isolation

The success of pathogen isolation and results of bioassay depend heavily on the quality of samples (time since collection and time in storage). Fresh specimens should be kept on ice and preferably sent to the laboratory within 24 hours of collection. Alternate storage methods should be used only after consultation with the receiving laboratory.

Before transfer to the laboratory, pieces of the organs to be examined should be removed from the fish with sterile dissection tools and transferred to sterile plastic tubes containing at least 4 ml transport medium, i.e. cell culture medium with 10% fetal calf serum (FCS) and antibiotics. The combination of 200 International Units (IU) penicillin, 200 µg streptomycin, and 200 µg kanamycin per ml are recommended, although other antibiotics of proven efficiency may also be used. The tissue in each sample should be larger than the analytical unit size required for initial laboratory testing (e.g. between 0.5 and 2 g) and taken in duplicate if retesting may be required.

Tubes containing fish tissues in transport medium for cell cultivation should be placed in insulated containers, such as thick-walled polystyrene boxes, together with sufficient ice or an alternative cooling medium with the similar cooling effect to ensure chilling of the samples during transportation to the laboratory. However, freezing of the samples should be avoided. The temperature of a sample during transit must never exceed 10°C.

Whole fish may be sent to the laboratory if the temperature requirements referred to in the first paragraph during transportation can be fulfilled. Whole fish should be wrapped up in paper with absorptive capacity and enclosed in a plastic bag. Live fish may also be transported to the laboratory.

The virological examination for isolation in cell culture should be started as soon as possible and no later than 48 hours after the collection of the samples. In exceptional cases, the virological examination may be started at the latest within 72 hours after the collection of the material, provided that the material to be examined is protected by a transport medium and that the temperature requirements during transportation can be fulfilled.

3.5.2. Preservation of samples for molecular detection

Samples can be taken from the fish in accordance with the procedure described in Section 3.5.1, using a sterile instrument, and transferred to a sterile plastic tube containing transport medium.

Alternatively, tissue samples for RT-PCR testing should be preserved in an appropriate medium for preservation of RNA. Samples in RNA stabilising reagents can be shipped on ice or at room temperature if transport time does not exceed 24 hours.

For further storage the samples can be kept at –20°C.

3.5.3. Fixed samples for histopathology, immunohistochemistry or *in-situ* hybridisation

Tissue samples for histopathology should be fixed in 10% neutral buffered formalin immediately after collection. The recommended ratio of fixative to tissue is 10:1.

3.5.4. Fixed samples for electron microscopy

Samples for electron microscopy are not routinely required and are collected only when it is considered beneficial to facilitate further diagnostic investigation. A 2 mm cubed section from each of the appropriate organs described in section 3.2 should be fixed in glutaraldehyde; the recommended ratio of fixative to tissue is 10:1.

3.5.5. Samples for other tests

Blood samples should be centrifuged for the collection of serum or plasma as soon as possible after sampling, to avoid lysis of the red blood cells. Serum or plasma samples should be shipped on ice to the laboratory to ensure virus viability.

3.6. Pooling of samples

The reliability of a virus isolation and real-time RT-PCR for detecting SAV in pooled samples from apparently healthy and clinically diseased populations of Atlantic salmon has been evaluated (Hall *et al.*,2014). The results suggest that the use of individual samples rather than pools is more appropriate when testing for freedom from, or for confirmatory diagnosis of, infection with SAV (Hall *et al.,* 2014).

4. Diagnostic methods

The methods currently available for identifying infection that can be used in i) surveillance of apparently healthy populations), ii) presumptive and iii) confirmatory diagnostic purposes are listed in Table 4.1. by life stage. The designations used in Table 4.1 indicate:

Key:

+++ =	Recommended method(s) validated for the purpose shown and usually to stage 3 of the OIE Validation Pathway;
++ =	Suitable method(s) but may need further validation;
+ =	May be used in some situations, but cost, reliability, lack of validation or other factors severely limits its application;
Shaded boxes =	Not appropriate for this purpose.

The selection of a test for a given purpose depends on the analytical and diagnostic sensitivities and specificities repeatability and reproducibility. OIE Reference Laboratories welcome feedback on diagnostic performance for assays, in particular PCR methods, for factors affecting assay analytical sensitivity or analytical specificity, such as tissue components inhibiting amplification, presence of nonspecific or uncertain bands, etc., and any assays that are in the +++ category.

Table 4.1. *OIE recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals*

Method	A. Surveillance of apparently healthy animals				B. Presumptive diagnosis of clinically affected animals				C. 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
Wet mounts												
Histopathology[3]					++	++	++					
Cytopathology[3]												
Cell or artificial media culture					+	+	+		+	+	+	
Real-time RT-PCR	+++	+++	+++		+++	+++	+++		+++	+++	+++	
Conventional RT-PCR					++	++	++		++	++	++	
Amplicon sequencing[4]									+++	+++	+++	
***In-situ* hybridisation**												
Bioassay												
LAMP												
Ab ELISA												
Ag ELISA												
Immunohistochemistry (needs to be filled in by expert)												
Serum neutralisation assay		+	++		++	++	++					

LV = level of validation, refers to the stage of validation in the OIE Pathway (chapter 1.1.2); RT-PCR = reverse transcription-polymerase chain reaction methods; LAMP = loop-mediated isothermal amplification; Ab- or Ag-ELISA = antibody or antigen enzyme-linked immunosorbent assay, respectively
[1]For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). [2]Early and juvenile life stages have been defined in Section 2.2.3.
[3]Histopathology and cytopathology can be validated if the results from different operators has been statistically compared. [4]Sequencing of the PCR product.
[5]Specify the test used. Shading indicates the test is inappropriate or should not be used for this purpose.

4.1. Wet mounts

Not relevant.

4.2. Histopathology and cytopathology

The pathological changes most commonly found in clinically diseased fish are severe loss of exocrine pancreatic tissue, cardiomyocytic necrosis and inflammation, red (aerobic) skeletal muscle inflammation and white (anaerobic) skeletal muscle degeneration or inflammation. A less frequent but supporting finding is the detection of cells with many cytoplasmic eosinophilic granules along kidney sinusoids.

As the disease progresses, the development of these changes is not simultaneous in all organs: in a very short, early phase, the only lesions present might be necrosis of exocrine pancreatic tissue and a variable inflammatory reaction in the peripancreatic fat. Shortly thereafter, heart muscle cell degeneration and necrosis develop before the inflammation response in the heart becomes more pronounced. The pancreatic necrotic debris will seemingly disappear, and the typical picture of severe loss of exocrine pancreatic tissue will soon appear simultaneously with the increasing inflammation in the heart. Somewhat later, skeletal muscle degeneration, inflammation and fibrosis develop. In a proportion of fish, severe fibrosis of the peri-acinar tissue may occur, and in these cases, the pancreas does not recover (runts) (Christie *et al.,* 2007; Kerbart Boscher *et al.,* 2006; McLoughlin & Graham, 2007; Taksdal *et al.,* 2007).

Cytopathology is not relevant for diagnostic use.

4.3. Cell or artificial media culture for isolation

4.3.1. Cell lines

Isolation of field isolates of SAV in cell culture may be challenging (Christie *et al.,* 1998; Graham *et al.,* 2007b; Petterson *et al.,* 2013). CHSE-214 are commonly used for primary SAV isolation, but susceptible cell lines such as BF-2, FHM, SHK-1, EPC, CHH-1 or others, may be used. Variation in cell line susceptibility among different SAV field isolates has been reported (Graham *et al.,* 2008; Herath *et al.,* 2009), and it is therefore recommended that several cell lines are tested for initial cell culture isolation of SAV in a new laboratory or for a new virus strain. Cell lines should be monitored to ensure that susceptibility to targeted pathogens has not changed.

The CHSE-214 cells are grown at 20°C in Eagle's minimal essential medium (EMEM) with non-essential amino acids and 0.01 M HEPES (N-2-hydroxyethyl-piperazine-N-2-ethanesulfonic acid) buffer, or Leibovitz's L-15 cell culture medium, both supplemented with fetal bovine serum (FBS) (5% or 10%) and L-glutamine (4 mM).

4.3.2. Sample preparation and inoculation

For virus isolation, cells are grown in tissue culture flasks or multi-well cell culture plates. SAV-positive controls may be inoculated in parallel with the tissue samples as a test for cell susceptibility to SAV. When positive controls are included, measures must be taken to avoid contamination.

i) Inoculation of cell monolayers

Prepare a 2% suspension of tissue homogenate or a 10% suspension of serum using L-15 medium or EMEM without serum, or other medium with documented suitability. Remove growth medium from actively growing monolayers (1- to 2-day-old cultures or cultures of 70–80% confluency) grown in tissue culture flasks or multi-well cell culture plates (see above). Inoculate monolayers with a low volume of the 2% tissue homogenate or 10% serum dilution (for 25 cm^2 flasks: 1.5 ml). Adjust volume to the respective surface area in use. Allow 2–3 hours of incubation at 15°C, followed by removal of the inoculum, and addition of fresh L-15 or EMEM medium supplemented with 2–5% fetal bovine serum (for 25 cm^2 flasks: 5 ml).

When fish samples come from production sites where IPNV is regarded as endemic, the tissue homogenate supernatant should be incubated (for a minimum of 1 hour at 15°C) with a pool of antisera to the indigenous serotypes of IPNV prior to inoculation.

ii) Monitoring incubation

Inoculated cell cultures (kept at 15°C) are examined at regular intervals (at least every 7 days) for the occurrence of cytopathic effect (CPE). Typical CPE due to SAV appears as plaques of pyknotic, vacuolated cells. However, Norwegian SAV field isolates (both SAV3 and SAV2) usually do not produce CPE in low passages, and this is also reported for other SAV genotypes (Graham *et al.*, 2008; Petterson *et al.*, 2013). If no CPE has developed after 14 days, subculture to fresh cell cultures.

iii) Subcultivation procedure

14 days (or earlier when obvious CPE appears) after inoculation, the cultures are freeze–thawed at –80°C to release virus from the infected cells. The procedure can be repeated 1–2 times.

Following centrifugation at 3000 ***g*** for 5 minutes, the supernatants are inoculated into fresh cell cultures as described for the primary inoculation: remove growth medium, inoculate monolayers with a small volume of diluted supernatant (1/5 and higher dilutions) for 2–3 hours before addition of fresh medium.

Inoculated cell cultures are incubated for at least 14 days and examined at regular intervals, as described for the primary inoculation. At the end of the incubation period, or earlier if obvious CPE appears, the medium is collected for virus identification, as described below. Cell cultures should always be examined for the presence of SAV by immunofluorescence (indirect fluorescent antibody test [IFAT]), as virus replication may occur without development of apparent CPE.

4.4. Nucleic acid amplification

4.4.1. Reverse-transcription, real-time polymerase chain reaction

The primers described below for real-time RT-PCR and RT-PCR with sequencing will detect all known genotypes of SAV.

RT-PCR may be used for detection of SAV from total RNA (or total nucleic acids) extracted from recommended organs or tissues (see Section 3.4). Real-time RT-PCR for the detection of SAV is recommended as it increases the specificity and also the sensitivity of the test.

For genotyping, RT-PCR with subsequent sequencing of fragments from the E2 gene is recommended.

The primers and probe sequences for real-time RT-PCR from the nsP1 gene, as well as primers for genotyping, are listed in Table 4.2. The E2-primers may also be used for conventional RT-PCR detection of SAV, if necessary. For RNA extraction, automatic and semi-automatic nucleic acid extractors can be used. In addition, a variety of manual RNA extraction kits can also be used successfully to extract SAV RNA. Various RT-PCR kits and qPCR machines can be used. The PCR programme depends on the kit and real-time PCR equipment used in the laboratory. The conditions for performing the real-time RT-PCR in the OIE Reference Laboratory is as follows: 50°C for 10 minutes, 95°C for 3 minutes, and 40 cycles of (95°C for 10 seconds, 60°C for 20 seconds). For the conventional RT-PCRs (sequencing), the following programme is used: 50°C for 30 minutes, 95°C for 15 minutes, and 45 cycles of (94°C for 60 seconds, 55°C for 45 seconds, 72°C for 60 seconds).

Table 4.2. *Primers and probe sequences for RT-PCR and real time RT-PCR*

Primer and probe sequences	Genomic segment	Product size	Reference
QnsP1F: 5'-CCG-GCC-CTG-AAC-CAG-TT-3' QnsP1R: 5'-GTA-GCC-AAG-TGG-GAG-AAA-GCT-3' QnsP1probe: 5'FAM-CTG-GCC-ACC-ACT-TCG-A-MGB3' (Taqman®probe)	QnsP1	107 nt	Hodneland *et al.*, 2006
E2F: 5'-CCG-TTG-CGG-CCA-CAC-TGG-ATG-3' E2R: 5'-CCT-CAT-AGG-TGA-TCG-ACG-GCA-G-	E2	516 nt	Fringuelli *et al.*, 2008

The following controls should be run with each assay: negative extraction control; positive template control; no template control.

4.4.2. Conventional PCR (PCR)

See Section 4.4.1. for comments on conventional PCR kits and PCR machines.

The following controls should be run with each assay: negative extraction control; positive template control; no template control.

4.4.3. Other nucleic acid amplification methods

Not applicable

4.5. Amplicon sequencing

Sequencing to determine the genotype of SAV can be performed using the E2-primer set listed in Table 4.2.

4.6. *In-situ* hybridisation

Not applicable

4.7. Immunohistochemistry

Immunohistochemical testing (Taksdal *et al.,* 2007) is only recommended for samples from fish with acute necrosis of exocrine pancreatic tissue.

4.7.1. Preparation of tissue sections

The tissues are fixed in neutral phosphate-buffered 10% formalin for at least 1 day, dehydrated in graded ethanol, cleared in xylene and embedded in paraffin, according to standard protocols. Approximately 3 µm thick sections (for immunohistochemistry sampled on poly-L-lysine-coated slides) are heated at 56–58°C (maximum 60°C) for 20 minutes, dewaxed in xylene, rehydrated through graded ethanol, and stained with haematoxylin and eosin for histopathology and immunohistochemistry as described below.

4.7.2. Staining procedure for immunohistochemistry

All incubations are carried out at room temperature and all washing steps are done with Tris-buffered saline (TBS).

i) Nonspecific antibody binding sites are first blocked in 5% bovine serum albumin (BSA) in TBS for 20 minutes. The solution is then poured off without washing.

ii) Sections are incubated with primary antibody (monoclonal mouse antibody 4H1 against E1 SAV glycoprotein [Todd *et al.,* 2001]), diluted 1/3000 in 2.5% BSA in TBS and then incubated overnight, followed by two wash out baths lasting a minimum of 5 minutes.

iii) Sections are incubated with secondary antibody (biotinylated rabbit anti-mouse Ig) diluted 1/300 for 30 minutes, followed by wash out baths as in step ii above.

iv) Sections are incubated with streptavidin with alkaline phosphatase 1/500 for 30 minutes followed by wash out baths as in step ii above.

v) For detection of bound antibodies, sections are incubated with Fast Red[6] (1 mg ml^{-1}) and Naphthol AS-MX phosphate (0.2 mg ml^{-1}) with 1 mM Levamisole in 0.1 M TBS (pH 8.2) and allowed to develop for 20 minutes followed by one wash in tap water before counterstaining with Mayer's haematoxylin and mounting in aqueous mounting medium.

[6] Reference to specific commercial products as examples does not imply their endorsement by the OIE. This applies to all commercial products referred to in this *Aquatic Manual*.

SAV-positive and SAV-negative tissue sections are included as controls in every setup (Taksdal *et al.,* 2007).

4.8. Bioassay

Not applicable.

4.9. Antibody or antigen-based detection methods

4.9.1. Antibody-based verification of SAV growth in cell culture

This technique should not be used as a screening method. All incubations below are carried out at room temperature unless otherwise stated.

i) Prepare monolayers of cells in appropriate tissue culture plates (e.g. 96-well plates) or on cover-slips, depending on the type of microscope available (an inverted microscope equipped with UV light is necessary for monolayers grown on tissue culture plates). The necessary monolayers for negative and positive controls must be included.

ii) Inoculate the monolayers with the virus suspensions to be identified in tenfold dilutions, two monolayers for each dilution. Add positive virus control in dilutions known to give a good staining reaction. Incubate inoculated cell cultures at 15°C for 9–11 days.

iii) Fix in 80% acetone for 20 minutes after removing cell culture medium and rinsing once with 80% acetone. Remove the fixative and air dry for 1 hour. If necessary, the fixed cell cultures may be stored dry for 14 days at 4°C until staining.

iv) Incubate the cell monolayers with anti-SAV MAb in an appropriate dilution in phosphate-buffered saline (PBS) for 1 hour and rinse three times with PBS with 0.05% Tween 20.

v) Incubate with fluorescein isothiocyanate (FITC)-conjugated anti-mouse immunoglobulin for 1 hour (or if the primary Ab is polyclonal from rabbits, use FITC-conjugated antibody against rabbit immunoglobulin), according to the instructions of the supplier. To increase the sensitivity of the test, FITC-conjugated anti-mouse Ig may be replaced with biotin-labelled anti-mouse Ig and FITC-labelled streptavidin with rinsing as in step d) in between the steps. The nuclei can be stained with propidium iodide (100 µg ml^{-1} in sterile distilled water). Add PBS (without Tween 20) and examine under UV light. To avoid fading, the stained plates should be kept in the dark until examination. For long periods of storage (more than 2–3 weeks) a solution of 1,4-diazabicyclooctane (DABCO 2.5% in PBS, pH 8.2) or similar reagent may be added as an anti-fade solution.

4.10. Other methods

4.10.1. Immunoperoxidase-based serum neutralisation assay

Experimental studies have shown that neutralising antibodies can first be detected 10–16 days post-infection (Graham *et al.,* 2003), and serum neutralisation (SN) assays can be used as a diagnostic tool for the detection of SAV antibodies. SN assays are based on the presence or absence of detectable virus growth in cultured cells following incubation with serum that may contain neutralising antibodies. In addition, the assay allows detection of virus in serum or plasma, if present.

CHSE-214 cells are grown as described in Section 4.3.1 Cell lines. A suspension of trypsinised cells, diluted 1/3 in growth medium (10% FBS) is prepared for the SN assay.

i) 1/20 and 1/40 dilutions of each test serum are prepared in maintenance medium (2% FBS), and transferred to two duplicate wells (15 µl per well) on a flat-bottomed tissue culture grade microtitre plate. An equal volume of virus (100 $TCID_{50}$ [median tissue culture infective dose]) is added and the plate is incubated for 2 hours at room temperature.

ii) 70 µl of maintenance medium, and 50 µl of the CHSE-214 cell suspension is added to each well, and the plates are incubated for 3 days at 15°C.

iii) The cell monolayer is then fixed and stained as described in Section 4.9.1 *Antibody-based verification of SAV growth in cell culture*, or using the following procedure: monolayers of CHSE-214 cells are fixed for 30 minutes at room temperature in 10% neutral buffered formalin. Following two washes with 0.01 M PBS, a MAb against SAV is added to the monolayers in an appropriate dilution. Bound MAb is visualised using a labelled streptavidin–biotin system according to the manufacturer's instructions.

iv) SN titres (ND_{50}) are then calculated according to the method of Karber (1931), with titres ≥ 1:20 being considered positive. Both serum controls (without virus added) and a virus control (without serum added) must always be included in the assay, to ensure valid results.

5. Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations

The recommended test to be used in surveillance of susceptible fish populations for declaration of freedom from SAV is real-time RT-PCR as described in Section 4.4.1. in this chapter.

6. Corroborative diagnostic criteria

This section only addresses the diagnostic test results for detection of infection in the absence (Section 6.1.) or in the presence of clinical signs (Section 6.2.) but does not evaluate whether the infectious agent is the cause of the clinical event.

The case definitions for a suspect and confirmed case have been developed to support decision making related to trade and confirmation of disease status at the country, zone or compartment level. Case definitions for disease confirmation in endemically affected areas may be less stringent. It is recommended that all samples that yield suspect positive test results in an otherwise pathogen-free country or zone or compartment should be referred immediately to the OIE Reference Laboratory for confirmation, whether or not clinical signs are associated with the case. If a laboratory does not have the capacity to undertake the necessary diagnostic tests it should seek advice from the appropriate OIE Reference Laboratory.

6.1. Apparently healthy animals or animals of unknown health status[7]

Apparently healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link(s) to an infected population. Geographic proximity to, or movement of animals or animal products or equipment, etc., from a known infected population equate to an epidemiological link. Alternatively, healthy populations are sampled in surveys to demonstrate disease freedom.

6.1.1. Definition of suspect case in apparently healthy animals

The presence of infection with SAV shall be suspected if at least one of the following criteria is met:

i) Positive result by real-time RT-PCR

ii) Positive result by conventional RT-PCR

iii) SAV-typical CPE in cell culture

iv) Detection of neutralising activity against SAV in serum or plasma.

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with SAV is considered to be confirmed if, in addition to the criteria in Section 6.1.1., one or more of the following criteria is met:

[7] For example transboundary commodities.

i) A positive result by real-time RT-PCR and a positive result by conventional RT-PCR and sequencing of the amplicon

ii) SAV-typical CPE in cell culture followed by virus identification by conventional RT-PCR and sequencing of the amplicon.

Reference Laboratories should be contacted for specimen referral when testing laboratories cannot undertake any of the recommended test methods and testing is being undertaken that will result in notification to the OIE.

6.2 Clinically affected animals

Clinical signs are not pathognomonic for a single disease; however, they may narrow the range of possible diagnoses.

6.2.1. Definition of suspect case in clinically affected animals

The presence of infection with SAV shall be suspected if at least one of the following criteria is met:

i) Gross pathology or clinical signs associated with infection with SAV

ii) Histopathology consistent with SAV infection

iii) Positive result by real-time RT-PCR

iv) Positive result by conventional RT-PCR

v) SAV-typical CPE in cell culture

vi) Detection of neutralising activity against SAV in serum or plasma.

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with SAV is considered to be confirmed if, in addition to the criteria Section 6.2.1., one of the following criteria is met.:

i) A positive result by real-time RT-PCR and a positive result by conventional RT-PCR and sequencing of the amplicon

ii) SAV-typical CPE in cell culture followed by virus identification by conventional RT-PCR and sequencing of the amplicon.

Reference Laboratories should be contacted for specimen referral when testing laboratories cannot undertake any of the recommended test methods and testing is being undertaken that will result in notification to the OIE.

6.3. Diagnostic sensitivity and specificity for diagnostic tests

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with SAV are provided in Table 6.3. This information can be used for the design of surveys for infection with SAV, however, it should be noted that diagnostic performance is specific to the circumstances of each diagnostic accuracy study (including the test purpose, source population, tissue sample types and host species) and diagnostic performance may vary under different conditions. Data is only presented where tests are validated to at least level two of the validation pathway described in Chapter 1.1.2. and the information is available within published diagnostic accuracy studies.

Table 6.1. *Diagnostic performance of tests recommended for surveillance or diagnosis*

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation
Real-time PCR	Diagnosis	Clinically diseased fish	Heart and mid-kidney	Atlantic salmon				Jansen et al, 2019
Isolation of SAV in cell culture	Diagnosis	Clinically diseased fish	Heart and mid-kidney	Atlantic salmon				Jansen et al, 2019
Detection of neutralising activity against SAV	Diagnosis	Clinically diseased fish	Serum or plasma	Atlantic salmon				Jansen et al, 2019
Histopathology	Diagnosis	Clinically diseased fish	Heart and mid-kidney	Atlantic salmon				Jansen et al, 2019

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = number of samples used in the study (this is pending further consultation with the expert), PCR: = polymerase chain reaction.

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NB: There is an OIE Reference Laboratory for infection with salmonid alphavirus
(see Table at the end of this *Aquatic Manual* or consult the OIE web site for the most up-to-date list:
http://www.oie.int/en/scientific-expertise/reference-laboratories/list-of-laboratories/).
Please contact the OIE Reference Laboratories for any further information on infection with salmonid alphavirus

NB: FIRST ADOPTED IN 2014. MOST RECENT UPDATES ADOPTED IN 2019