Annex 29. Item 10.1.3. Chapter 2.4.7. ‘Infection with *Xenohaliotis californiensis’*

Chapter 2.4.7

infection with
*Xenohaliotis Californiensis*

1. Scope

Infection with *Xenohaliotis californiensis* means infection with the pathogenic agent *Candidatus* Xenohaliotis californiensis of the Family Anaplasmataceae*.* For the purposes of this chapter, the pathogenic agent will be referred to as *Xenohaliotis californiensis.*

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

*Xenohaliotis californiensis* is an intracellular bacterium in the family Anaplasmataceae (Dumler *et al.,* 2001) and is closely related to members of the genera *Ehrlichia, Anaplasma* and *Cowdria* (Friedman *et al.,* 2000). The disease caused by this bacterium is known as withering syndrome (Friedman *et al.,* 2002; Haaker *et al.,* 1992) and may be more appropriately termed abalone rickettsiosis. Some *X. californiensis* may be infected with a phage (Friedman & Crosson, 2012). The dimorphic rod-to-spherical-shaped bacterium measures an average of 332 × 1550 nm in the bacillus form and an average of 1405 nm in the spherical morphotype. The bacterium reproduces within intracytoplasmic vacuoles 14–56 µm in diameter within gastrointestinal epithelia (Friedman *et al.,* 2000).

2.1.2. Survival and stability in processed or stored samples

As the pathogen has not been cultured the survival and stability in stored samples is unknown.

2.1.3. Survival and stability outside the host

Although *X. californiensis* is thought to be an obligate intracellular organism, the bacterium may survive outside the host for an undetermined period of time as evidenced by water-borne transmission studies (Balseiro *et al*., 2006; Braid *et al*., 2005; Friedman *et al*., 2002; 2007; Rosenblum *et al*., 2008).

For inactivation methods, see Section 2.4.5.

2.2. Host factors

NB: Sections 2.2.1 and 2.2.2 are not for Member comment. Version for comment and adoption are presented in Annex 27

~~2.2.1. Susceptible host species~~

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

| **~~Family~~** | **~~Scientific name~~** | **~~Common name~~** |
| --- | --- | --- |
| ~~Haliotidae~~ | *~~Haliotis corrugata~~* | ~~pink abalone~~ |
| *~~Haliotis cracherodii~~* | ~~black abalone~~ |
| *~~Haliotis discus discus~~* | ~~Japanese abalone~~ |
| *~~Haliotis diversicolor~~* | ~~small abalone~~ |
| *~~Haliotis fulgens~~* | ~~green abalone~~ |
| *~~Haliotis kamtschatkana~~* | ~~pinto abalone~~ |
| *~~Haliotis rufescens~~* | ~~red abalone~~ |
| *~~Haliotis rufescens~~* ~~X~~ *~~Haliotis discus hannai~~* ~~hybrid~~ | ~~hybrid red and Japanese abalone~~ |
| *~~Haliotis sorenseni~~* | ~~white abalone~~ |
| *~~Haliotis tuberculata~~* | ~~tuberculate abalone~~ |

~~2.2.2. Species with incomplete evidence for susceptibility~~

*~~Haliotis gigantea~~* ~~has incomplete evidence to fulfil the criteria for listing as susceptible to infection with X. californiensis according to Chapter 1.5.~~

~~In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in~~ *~~Haliotis discus hannai~~*~~, but no active infection has been demonstrated.~~

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

The bacterium divides by binary fission (Friedman *et al.,* 2000) and has direct, horizontal transmission (Braid *et al*., 2005; Friedman *et al*., 2002; Moore *et al*., 2001). Although not typically observed in farmed abalones until they are in grow-out conditions (>2.5 cm maximum size), polymerase chain reaction (PCR) examination of exposed 6-week-old abalones suggested that 1–2 mm abalones may become infected (Moore *et al*., unpublished observations). Probability of detection increases with increasing abalone size. Animals less than 10 mm in size have a reduced probability of detection using histology but equal probability of detection using PCR (Friedman *et al*., 2007; Moore *et al*., 2011).

While all post-larval life stages have been demonstrated susceptible to infection with *X. californiensis*, clinical disease is typically observed in animals >1 years of age in farmed abalones (Friedman, unpublished observations) and all abalone size classes observed in wild populations surveyed to date (e.g. Balseiro *et al*., 2006; Braid *et al*., 2005; Friedman *et al*., 1997; Haaker *et al*., 1992; Steinbeck *et al*., 1992; Van Blaricom *et al*., 1993).

2.2.4. Distribution of the pathogen in the host

*Xenohaliotis californiensis* infects the gastrointestinal epithelial cells of the posterior oesophagus, digestive gland and, to a lesser extent, intestine (Friedman *et al*., 2000).

2.2.5. Aquatic animal reservoirs of infection

None.

2.2.6. Vectors

None.

2.3. Disease pattern

2.3.1. Mortality, morbidity and prevalence

Susceptibility varies with species as the bacterium is known to cause disease in *H. cracherodii* (up to 99% mortality; Moore *et al*., 2009), *H. sorenseni* (up to 100% mortality; Friedman & McCormick, unpublished observations), *H. rufescens* (up to 35% mortality; Moore *et al*., 2000; 2001), *H. corrugata* and *H. fulgens* (Tinajero *et al*., 2002). Unlike the other abalone species studied to date, the magnitude of abalone mortality is not well documented in *H. corrugata* and *H. fulgens*. However, in Baja California, Mexico, up to 100% of *H. fulgens* and 63% of *H. corrugata* may be infected, with up to 43% of *H. fulgens* and 71% of *H. corrugata* having microscopic signs of disease (degenerated or metaplastic digestive gland; Tinajero *et al*., 2002).

The incubation period varies with temperature but typically involves a prolonged 3- to 7-month prepatent period. Mortality typically occurs 1–2.5 months after the onset of visible clinical signs (Friedman *et al*., 1997). Prevalence has not been well documented but up to 61% of *H. diversicolor supertexta* were infected at a farm in Thailand, however, like the European abalone, *H. tuberculata*, no abalones exhibited clinical signs of withering syndrome (Balseiro *et al*., 2001).

Infections may persist for long periods without the development of clinical disease when the host is maintained at cool water temperatures (e.g. 15°C for *H. rufescens*), and exposure to elevated seawater temperatures (e.g. >17°C for *H. rufescens*, *H. cracherodii* and *H. sorenseni*) typically results in clinical disease (Friedman & Finley, 2003; Moore *et al*., 2000; Steinbeck *et al*., 1992). Varying seawater temperatures with a lower mean temperature (e.g. 16.5°C for *H. rufescens*) may exacerbate losses (Moore *et al*., 2011). There is some suggestion that species, especially those inhabiting warmer waters may harbour the bacterium without the development of clinical disease (Wetchateng, 2008; Wetchateng *et al*., 2010).

2.3.2. Clinical signs, including behavioural changes

This intracellular pathogen infects the gastrointestinal epithelial cells, leading to clinical signs of starvation, including pedal and digestive gland atrophy. Abalones with *X. californiensis* infections may be sub-clinically infected during the prepatent period or at water temperatures ≤15°C. Infected individuals may be slightly to severely emaciated (atrophied) under permissive water temperatures.

During an epidemic, affected abalones will often cling to horizontal (as opposed to vertical or inverted) substrates and appear weak (easily removed from the substrate by hand) and emaciated (withered) (Haaker *et al*., 1992). Farmed abalones will also be anorexic. In addition, the presence of an abnormally high number of fresh shells may also indicate disease.

2.3.3 Gross pathology

Clinical disease is characterised by morphological changes in the digestive gland, which vary between species and may include degeneration (atrophy of tubules, increase in connective tissues and inflammation) and/or metaplasia of the digestive tubules. Metaplasia involves the replacement of terminal secretory/absorptive acini with absorptive/transport ducts similar in appearance to the post-oesophagus. These morphological changes are accompanied by anorexia, depletion of glycogen reserves, followed by use of the foot muscle as an energy source and subsequent death (Balseiro *et al*., 2006; Braid *et al*., 2005; Friedman *et al*., 1997; 2007; Kismohandaka *et al*., 1995; Moore *et al*., 2000; 2001). The foot of affected abalones contains fewer and less organised muscle bundles, abundant connective tissue and may contain more cerous cells than unaffected individuals (Friedman *et al*., 2007; Moore *et al*., 2000; Van Blaricom *et al*., 1993). Surviving abalones appear to remain infected, even in low water temperature environments, such as in northern California (Friedman & Finley, 2003).

2.3.4. Modes of transmission and life cycle

Transmission of *X. californiensis* is horizontal and is postulated to be via a faecal–oral route. Exposure of abalones to seawater containing infectious material is sufficient for transmission of the bacterium, and no direct animal contact is required (Balseiro *et al*., 2006; Braid *et al*., 2005; Friedman *et al*., 2002; Moore *et al*., 2000; 2001). Temperatures below 13°C have been demonstrated to limit transmission of the bacterium (i.e. less than 1% transmission) relative to those held at ~18°C (72–94% transmission) (Braid *et al*., 2005).

2.3.5. Environmental factors

Disease (withering syndrome) occurs at elevated water temperatures (~18–25°C in abalones with moderate to severe infections (Braid *et al*., 2005; Friedman *et al*., 2000; 2002; Moore *et al*., 2000; 2011; Rosenblum *et al*., 2008). Parasite transmission is enhanced in fed (94%) as opposed to starved. (72%) abalones (Balseiro *et al*., 2006; Braid *et al*., 2005). Subclinical infections have been observed in *H. diversicolor supertexta* raised at 27–29°C. As abalones are obligate marine species, salinity tolerances of the Rickettsia-like organism (RLO) have not been investigated.

2.3.6. Geographical distribution

*Xenohaliotis californiensis* occurs along the south-west coast of North America. However, as infected abalones have been transported to South America, Asia-Pacific, and Europe and possibly other regions, the geographical range of the aetiological agent is suspected to be broad where California red abalones, *Haliotis rufescens*, are cultured or areas where native species have been exposed to red abalones (e.g. Wetchateng, 2008)

See WAHIS (<https://wahis.woah.org/#/home>) for recent information on distribution at the country level.

2.4. Biosecurity and disease control strategies

2.4.1. Vaccination

No vaccines are available.

2.4.2. Chemotherapy including blocking agents

Reducing densities and application of an oxytetracycline-medicated diet may reduce losses (Friedman *et al*., 2003; 2007; Rosenblum *et al*., 2008). Oral administration of 12–19% TM-100 (90–100 mg kg–1) in a medicated diet for 10 or 20 days provides protection against bacterial re-infection for several months. A single day oral administration of 12% TM-100 may reduce bacterial infections from 80% to 10% prevalence and mean infection intensity from 1.4 to 0.1 on a scale of 0–3 (Friedman *et al*., 2000; 2007).

2.4.3. Immunostimulation

No immunostimulants are currently known

2.4.4. Breeding resistant strains

Interest in selecting for resistant abalones, particularly for restoration purposes, is increasing. Wild black abalones have continued to recruit along the California Channel Islands since 2002 and some recruits survive, suggesting that these individuals may be more resistant to this rickettsial disease (Tinajero *et al*., 2002).

2.4.5. Inactivation methods

Based on successful decontamination in the laboratory, this bacterium is readily inactivated by immersion in <10% bleach. In addition, exposure of seawater containing the bacterium to >10 mg litre–1 [ppm] calcium hypochlorite and disinfection of equipment in a bath of 1% tamed iodine in freshwater for 1 hour are effective disinfectants based on the use of these disinfection methods at a marine laboratory with flow-through seawater and a lack of detection of this pathogen in adjacent abalone populations (Friedman & Finley, 2003).

2.4.6. Disinfection of eggs and larvae

No attempts to disinfect eggs and larvae have been undertaken.

2.4.7. General husbandry

Husbandry practices to control *X. californiensis* are typical of those for any bacterial disease and include the purchase of inspected seed (devoid of evidence of infection), maintaining separate families or groups (i.e. avoid high grading and mixing of disparate groups), rinsing hands and equipment in freshwater or iodinated water and drying them in between uses. Isolation of infected groups is recommended if possible.

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

To optimise detection (targeted sampling), selection of abalones exhibiting the clinical sign of reduced weight (atrophied pedal muscle) is recommended. If possible, animals should be sampled after exposure to a period (e.g. 30 days) of warm water (e.g. >18°C).

3.2. Selection of organs or tissues

The best target tissue is the posterior oesophagus, and the second-best tissue is the digestive gland/intestine complex.

3.3. Samples or tissues not suitable for pathogen detection

Non-digestive tissues do not contain rickettsial DNA and should be avoided.

3.4. Non-lethal sampling

None

3.5. Preservation of samples for submission

3.5.1. Samples for pathogen isolation

Not applicable.

3.5.2. Preservation of samples for molecular detection

Tissue samples for PCR testing should be preserved in 80% (v/v) analytical-grade 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. If material cannot be fixed it may be frozen.

Standard sample collection, preservation and processing methods for molecular techniques can be found in Section B.5.5 of Chapter 2.4.0 *General information* (diseases of molluscs).

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

Standard sample collection, preservation and processing methods for histological techniques can be found in Section B.5.3 of Chapter 2.4.0 *General information* (diseases of molluscs).

3.5.4. Samples for other tests

None.

3.6. Pooling of samples

Pooling of samples from more than one individual animal for a given purpose is only recommended where robust supporting data on diagnostic sensitivity and diagnostic specificity have been evaluated and found to be suitable. If the effect of pooling on diagnostic sensitivity has not been thoroughly evaluated, specimens should be processed and tested individually. Small life stages such as spat can be pooled to obtain the minimum amount of material for virus isolation or molecular detection.

4. Diagnostic methods

The methods currently available for pathogen detection that can be used in i) surveillance of apparently healthy animals, ii) presumptive diagnosis in clinically affected animals and iii) confirmatory diagnostic purposes are listed in Table 4.1. by animal life stage.

**Ratings for purposes of use.** For each recommended assay a qualitative rating for the purpose of use is provided. The ratings are determined based on multiple performance and operational factors relevant to application of an assay for a defined purpose. These factors include appropriate diagnostic performance characteristics, level of assay validation, availability cost, timeliness, and sample throughput and operability. For a specific purpose of use, assays are rated as:

+++ = Methods are most suitable with desirable performance and operational characteristics.

++ = Methods are suitable with acceptable performance and operational characteristics under most circumstances.

+ = Methods are suitable, but performance or operational characteristics may limit application under some circumstances.

Shaded boxes = Not appropriate for this purpose.

**Validation stage**. The validation stage corresponds to the assay development and validation pathway in chapter 1.1.2. The validation stage is specific to each purpose of use. Where available, information on the diagnostic performance of recommended assays is provided in Section 6.3.

WOAH Reference Laboratories welcome feedback on diagnostic performance of recommended assays, in particular PCR methods. Of particular interest are any factors affecting expected assay sensitivity (e.g. tissue components inhibiting amplification) or expected specificity (e.g. failure to detect particular genotypes, detection of homologous sequences within the host genome). These issues should be communicated to the WOAH Reference Laboratories so that advice can be provided to diagnostic laboratories and the standards amended if necessary.

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

| Method | 1. Surveillance of apparently healthy animals
 | 1. Presumptive diagnosis of clinically affected animals
 | 1. Confirmatory diagnosis1 of a suspect result from surveillance or presumptive diagnosis
 |
| --- | --- | --- | --- |
| Early life stages2 | Juveniles2 | Adults | LV | Early life stages2 | Juveniles2 | Adults | LV | Early life stages2 | Juveniles2 | Adults | LV |
| Wet mounts |  |  |  |  |  |  |  |  |  |  |  |  |
| Histopathology | + | ++ | ++ | 1 | + | +++ | +++ | 1 |  |  |  |  |
| Cell cultures |  |  |  |  |  |  |  |  |  |  |  |  |
| Real-time PCR | +++ | +++ | +++ | 3 | +++ | +++ | +++ | 3 | +++ | +++ | +++ | 3 |
| Conventional PCR | +++ | +++ | +++ | 1 | +++ | +++ | +++ | 1 |  |  |  |  |
| Conventional PCR followed by amplicon sequencing |  |  |  |  |  |  |  |  | +++ | +++ | +++ | 1 |
| *In-situ* hybridisation |  |  |  |  | + | + | + | 1 | + | + | + | 1 |
| Bioassay |  |  |  |  |  |  |  |  |  |  |  |  |
| LAMP |  |  |  |  |  |  |  |  |  |  |  |  |
| Ab-ELISA |  |  |  |  |  |  |  |  |  |  |  |  |
| Ag-ELISA |  |  |  |  |  |  |  |  |  |  |  |  |
| Other antigen detection methods3 |  |  |  |  |  |  |  |  |  |  |  |  |
| Other methods3 |  |  |  |  |  |  |  |  |  |  |  |  |

LV = level of validation, refers to the stage of validation in the WOAH 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, respectively; IFAT = indirect fluorescent antibody test. [give definitions of abbreviations as appropriate; nPCR = nested PCR, etc. NB “RT-PCR” is reserved for reverse-transcription polymerase chain reaction methods. “real-time PCR” should always be stated in full and refers to probe-based and SYBR green assays]
1For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). 2Susceptibility of early and juvenile life stages is described in Section 2.2.3.
3Specify the test used. Shading indicates the test is inappropriate or should not be used for this purpose.

4.1. Wet mounts

Not applicable.

4.2. Histopathology and cytopathology

The presence of basophilic, ovoid intracytoplasmic bacterial inclusions in digestive epithelia (posterior oesophagus, transport ducts and metaplastic epithelia of the digestive gland, and/or intestine). Metaplastic changes in the digestive gland that include the transformation of the terminal digestive tubules into absorptive/transport epithelia occurs in abalones infected with *X. californiensis* (Balseiro *et al*., 2006; Braid *et al*., 2005; Friedman *et al*., 2002; Moore *et al*., 2000; 2001). Although metaplasia has been observed in all affected species examined to date, the response to infection may vary between hosts. Red abalones and white abalones, for example, typically respond with a metaplastic change (Balseiro *et al*., 2006; Braid *et al*., 2005; Moore *et al*., 2000), while black abalones generally respond with a combination of metaplasia, digestive tubule degeneration and inflammation (Friedman *et al*., 1997; 2002). Affected individuals contain less pedal glycogen and fewer muscle bundles than do unaffected individuals (Balseiro *et al*., 2006; Braid *et al*., 2005; Gardner *et al*., 1995). In some abalones, an increase in serous cells may be observed in the foot muscle (Van Blaricom *et al*., 1993), but these signs are not pathognomonic for this disease. Juvenile white abalone may contain an apparently metaplastic digestive without presence of *X. californiensis* (Friedman *et al*., 2007). Thus, the presence of *X. californiensis* in digestive epithelia in conjunction with the morphological changes noted above indicate the presence of clinical withering syndrome.

4.3. Cell culture for isolation

Not applicable.

4.4. Nucleic acid amplification

PCR assays should always be run with the controls specified in Section B.5.5 *Molecular methods* Chapter 2.4.0 *General information* (diseases of molluscs). Each sample should be tested in duplicate.

*Extraction of nucleic acids*

Different kits and procedures can be used for nucleic acid extraction. The quality and concentration of the extracted nucleic acid is important and can be checked using a suitable method as appropriate to the circumstances.

4.4.1. Real-time PCR

Method 1: Friedman *et al.,* 2014; GenBank Accession No.: AF133090, amplicon size: 147 bp

|  |  |  |  |
| --- | --- | --- | --- |
| Pathogen/target gene | Primer/probe (5’–3’) | Concentration | Cycling parameters |
| 16S rDNA | Fwd WSN1 F: AGT-TTA-CTG-AAG-GCA-AGT-AGC-AGARev WSN1 R: TCT-AAC-TTG-GAC-TCA-TTC-AAA-AGCProbe WS-RLO\_P: TGC-TTG-GAA-ATC-TAC-TCA-GAA-GAC-ATG-A | 320 nM320 nM200 nM | 45 cycles of: 95°C/15 sec and 60°C/30 sec |

4.4.2. Conventional PCR

Method 1: Andree *et al*.*,* 2000; GenBank Accession No.: AF133090, amplicon size: 160 bp

Method 2: Cicala *et al*.*,* 2017; GenBank Accession No.: KU645900, amplicon size: 426 bp

|  |  |  |  |
| --- | --- | --- | --- |
| Pathogen/target gene | Primer (5’–3’) | Concentration | Cycling parameters(a) |
| 16S rDNAMethod 1 | Fwd RA 5-1: GTT-GAA-CGT-GCC-TTC-AGT-TTA-CRev RA 3-6**:** ACT-TGG-ACT-CAT-TCA-AAA-GCG-GA | 500 nM500 nM | 40 cycles of: 95°C/60 sec, 50°C/30 sec and 72°C/30 sec |
| 16S rDNAMethod 2 | Fwd ss16S-F: GCC-TCA-GTT-TGG-CTG-GGT-TCT-TCARev ss16S-R: GAA-TTG-CCA-CTT-TAA-AGT-ATG-GAC-GG | 300 nM300 nM | 40 cycles of: 94°C/60 sec, 66°C/30 sec and 72°C/30 sec |

4.4.3. Other nucleic acid amplification methods

Not applicable.

4.5. Amplicon sequencing

The size of the PCR amplicon should be verified, for example by agarose gel electrophoresis. Both DNA strands of the PCR product must be sequenced and analysed in comparison with reference sequences.

4.6. *In-situ* hybridisation

Use of Rickettsiales-like prokaryotes specific DNA probes with histological sections is useful to demonstrate the presence of *X. californiensis* nucleic acid in infected cells (Antonio *et al*., 2000). See Chapter 2.4.0 Section 5.5.4 for general comments on *in-situ* hybridisation.

Antonio *et al.* (2000) developed an ISH method targeting the 16S rDNA gene. This method allows the detection of Rickettsiales-like prokaryotes in tissue sections. Although this method has not been formally validated, tests for specificity using several bivalve and fish rickettsial organisms suggested that the test was specific for *X. californiensis*.

|  |  |  |  |
| --- | --- | --- | --- |
| Reference | Pathogen/target gene | ISH probe | Probe size |
| Antonio *et al.* (2000) | 16S rDNA | RA 5-1: GTT-GAA-CGT-GCC-TTC-AGT-TTA-CRA 3-6: ACT-TGG-ACT-CAT-TCA-AAA-GCG-GA; RA 3-8: CCA-CTG-TGA-GTG-GTT-ATC-TCC-TG; RA 5-6: GAA-GCA-ATA-TTG-TGA-GAT-AAA-GCA. | oligo nucleotide probes |

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

The recommended method for surveillance is real-time PCR using the assay by Friedman *et al.* (2014).

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. If a Competent Authority does not have the capability to undertake the necessary diagnostic tests it should seek advice from the appropriate WOAH Reference Laboratory, and if necessary, refer samples to that laboratory for confirmatory testing of samples from the index case in a country, zone or compartment considered free. There are currently no WOAH Reference Laboratories for *Xenohaliotis californiensis*.

6.1. Apparently healthy animals or animals of unknown health status[[1]](#footnote-2)

Apparently healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link(s) to an infected population. Hydrographical 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 *X. californiensis* shall be suspected if at least one of the following criteria is met:

i) Histopathological changes consistent with the presence of the pathogen or the disease

ii) Positive result by conventional PCR

iii) Positive result by real-time PCR

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with *X. californiensis* is considered to be confirmed if at least one of the following criteria is met:

i) Positive result by real-time PCR and positive result by conventional PCR followed by amplicon sequencing

ii) Positive result by *in-situ* hybridisation and positive result by conventional PCR followed by amplicon sequencing

iii) Positive result by *in-situ* hybridisation and positive result by real-time 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 *X. californiensis* shall be suspected if at least one of the following criteria is met:

i) Histopathological changes consistent with the presence of the pathogen or the disease

ii) Positive result by *in-situ* hybridisation

iii) Positive result by conventional PCR

iv) Positive result by real-time PCR

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with *X. californiensis* is considered to be confirmed if at least at least one of the following criteria is met:

i) Positive result by real-time PCR and positive result by conventional PCR followed by amplicon sequencing.

ii) Positive result by *in-situ* hybridisation and positive result by conventional PCR followed by amplicon sequencing.

iii) Positive result by *In-situ* hybridisation and positive result by real-time PCR.

6.3. Diagnostic sensitivity and specificity for diagnostic tests

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with *Xenohaliotis californiensis* are provided in Tables 6.3.1. and 6.3.2). Data are only presented where tests are validated to at least level 2 of the validation pathway described in Chapter 1.1.2. and the information is available within published diagnostic accuracy studies.

6.3.1. For presumptive diagnosis of clinically affected animals

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Test type | Test purpose | Source populations | Tissue or sample types | Species | DSe (*n*) | DSp (*n*) | Reference test | Citation |
| Real-time PCR | Diagnosis  | Clinically diseased abalone from farms | Posterior esophagus and digestive gland tissue | *Haliotis rufescens, H. sorensoni, H. fulgens, H. discushannai, H. cracherodii, H. kamtschatkana* | 100 (518) | 99.7 (518) | Histopathology | Friedman *et al.* (2014) |

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = number of animals used in the validation study,
PCR: = polymerase chain reaction.

6.3.2. For surveillance of apparently healthy animals (no data are currently available)

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Test type | Test purpose | Source populations | Tissue or sample types | Species | DSe (*n*) | DSp (*n*) | Reference test | Citation |
|  |  |  |  |  |  |  |  |  |

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = number of animals used in the validation study,
PCR: = polymerase chain reaction.

7. References

Andree K.B., Friedman C.S., Moore J.D. & Hedrick R.P. (2000). A polymerase chain reaction for detection of genomic DNA of a Rickettsiales-like prokaryote associated with Withering Syndrome in Black Abalone (*Haliotis cracherodii*). *J. Shellfish Res*., **19**, 213–218.

Antonio D.B., Andree K.B., Moore J.D., Friedman C.S. & Hedrick R.P. (2000). Detection of Rickettsiales-like prokaryotes (RLPs) by *in situ* hybridization in black abalone *Haliotis cracherodii* with withering syndrome. *J. Invertebr. Pathol.*, **75**, 180–182.

Balseiro P., Aranguren R., Gestal C., Novoa B. & Figueras A. (2006). *Candidatus* Xenohaliotis californiensis and *Haplosporidium montforti* associated with mortalities of abalone *Haliotis tuberculata* cultured in Europe. *Aquaculture*, **258**, 63–72.

Braid B.A., Moore J.D., Robbins T.T., Hedrick R.P., Tjeerdema R.S. & Friedman C.S. (2005). Health and survival of red abalone, *Haliotis rufescens*, under varying temperature, food supply and exposure to the agent of withering syndrome. *J. Invertebr. Pathol.,* **89**, 219–231.

Cicala F., Moore J.D., Caceres-Martinez J., Del Rio-Portilla M.A., Hernandex-Rodrigues M., Vasquez\_Yeomans R. & Rocha-Olivares A. (2017). Multigenic characterisation of “*Çandidatus* Xenohaliotis californiensis”. *Int. J. Syst. Evol. Microbiol.*, **67**, 42–49.

Dumler J.S., Barbet A.F., Bekker C.P.J., Dasch G.A., Palmer G.H., Ray S.C., Rikihisa Y. & Rurangirwa F.R. (2001). Reorganization of general in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and ‘HGE agent’ as subjective synonyms of E*hrlichia phagocytophila. Int. J. System. Evol. Microbiol*., **51**, 2145–2165.

Friedman C.S., Andree K.B., Beauchamp K.A., Moore J.D., Robbins T.T., Shields J.D. & Hedrick R.P. (2000). “*Candidatus* Xenohaliotis californiensis” a newly described pathogen of abalone, *Haliotis* spp., along the west coast of North America. *Int. J. Syst. Evol. Microbiol.,* **50**, 847–855.

Friedman C.S., Biggs W., Shields J.D. & Hedrick R.P. (2002). Transmission of withering syndrome in black abalone, *Haliotis cracherodii* Leach. *J. Shellfish Res*., **21**, 817–824.

Friedman C.S. & Crosson L.M. (2012). Putative phage hyperparasite in the Rickettsial Pathogen of Abalone, “*Candidatus* Xenohaliotis californiensis”. *Invertebr. Microbiol*., **64**, 1064–1072.

Friedman C.S. & Finley C.A. (2003). Evidence for an anthropogenic introduction of “*Candidatus* Xenohaliotis californiensis”, the etiological agent of withering syndrome, into northern California abalone populations via conservation efforts. *Can. J. Fish Aquat. Sci.,* **60**, 1424–1431.

Friedman C.S., Thomson M., Chun C., Haaker P.L. & Hedrick, R.P. (1997). Withering syndrome of the black abalone, *Haliotis cracherodii* (Leach): Water temperature, food availability, and parasites as possible causes. *J. Shellfish Res.*, **16**, 403–411.

Friedman C.S., Trevelyan G., Mulder E.P. & Fields R. (2003). Development of an oral administration of oxytetracycline to control losses due to withering syndrome in cultured red abalone *Haliotis rufescens. Aquaculture*, **224**, 1–23.

Friedman C.S., Scott B.B., Strenge R.E. & McCormick T.B. (2007). Oxytetracycline as a tool to manage and prevent losses of the endangered white abalone, *Haliotis sorenseni,* due to withering syndrome. *J. Shellfish Res*., **26**, 877–885.

Friedman C.S., Wight N., Crosson L.M., White S.J. & Strenge R.M. (2014). Validation of a qualitative PCR assay for detection and quantification of ‘*Candidatus* Xenohaliotis californiensis’. *Dis. Aquat. Organ*.,**108**, 251–259.

Gardner G.R., Harshbarger J.C., Lake J., Sawyer T.K., Price K.L., Stephenson M.D., Haaker P.L. & Togstad H.A. (1995). Association of prokaryotes with symptomatic appearance of withering syndrome in black abalone *Haliotis craherodii. J. Invertebr. Pathol.*, **66**, 111–120.

Haaker P.L., Parker D.O., Togstad H., Richards D.V., Davis G.E. & Friedman C.S. (1992). Mass mortality and withering syndrome in black abalone *Haliotis cracherodii*, in California. *In:* Abalone of the World, Shepard S.A., Tegner M.J. & Guzman del Proo S.A., eds, Blackwell Scientific, Oxford, UK, 214–224.

Kismohandaka G., Roberts W., Hedrick R.P. & Friedman C.S. (1995). Physiological alterations of the black abalone, *Haliotis cracherodii* Leach, with withering syndrome. *J. Shellfish Res*., **14**, 269–270.

Moore J.D., Juhasz C.I., Robbins T.T. & Vilchis I. (2009). Green abalone, *Haliotis fulgens*, infected with the agent of withering syndrome do not express disease signs under a temperature regime permissive for red abalone, *Haliotis rufescens*. *Marine Biol.,* **156**, 2325–2330.

Moore J.D., Marshman B.C. & Chun C.C. (2011). Health and survival of red abalone *Haliotis rufescens* from San Miguel Island, California, USA, in a laboratory simulation of La Niña and El Niño conditions. *J. Aquat. Anim. Health*, **23**, 78–84.

Moore J.D., Robbins T.T. & Friedman C.S. (2000). Withering syndrome in farmed red abalone *Haliotis rufescens:* Thermal induction and association with a gastrointestinal rickettsia-like prokaryote. *J. Aquat. Anim. Health,* **12**, 26–34.

Moore J.D., Robbins T.T., Hedrick R.P. & Friedman C.S. (2001) Transmission of the Rickettsiales-like procaryote “*Candidatus* Xenohaliotis californiensis” and its role in withering syndrome of California abalone *Haliotis* spp. *J. Shellfish Res.*, **20**, 867–874.

Rosenblum E.S., Juhasz C., Friedman C.S., Robbins T.T., Craigmill A., Tjeerdema R.S. & Moore J.D. (2008). Oxytetracycline as a treatment for abalone withering syndrome Part II: Efficacy, pharmacokinetics, and long term resistance to re-infection at elevated sea water temperatures. *Aquaculture*, **277**, 138–148.

Steinbeck J.R., Groff J.M., Friedman C.S., McDowell T. & Hedrick R.P. (1992). Investigations into mortality among populations of the California black abalone, *Haliotis cracherodii,* on the central coast of California, USA. *In:* Abalone of the World, Shepard S.A., Tegner M.J. & Guzman del Proo S.A., eds. Blackwell Scientific, Oxford, UK, 203–213.

Tinajero M.D.C.A., Caceres-Martinez J., & Aviles J.G.G. (2002) Histopathological evaluation of the yellow abalone *Haliotis corrugata* and the blue abalone *Haliotis fulgen*s from Baja California, Mexico. *J. Shellfish Res*., **21**, 825–830.

Van Blaricom G.R., Ruediger J.L., Friedman C.S., Woodard D.D. & Hedrick R.P. (1993). Discovery withering syndrome among black abalone populations at San Nicolas Island, California *J. Shellfish Res.*, **12**, 185–188.

Wetchateng T. (2008). *Rickettsia*-like organism (RLO) infection in the abalone *Haliotis diversicolor supertexta*: Histopathology, diagnosis and treatment. PhD. Dissertation, Mahidol University, Bangkok, Thailand, 49 pp.

Wetchateng T., Friedman C.S., Wight N.A., Lee P.-Y., Teng P.H., Sriurairattana S., Wongprasert K. & Withyachumnarnkul B. (2010). Withering syndrome in abalone *Halioits diversicolor supertexta*. *Dis. Aquat. Organ.,* **90**, 69–76.

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**NB:** Currently (2025) there is no WOAH Reference Laboratory for infection with *Xenohaliotis californiensis*
(please consult the WOAH web site:
<https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3>).

**NB:** First adopted in 2006. Most recent updates adopted in 2012.

1. For example transboundary commodities. [↑](#footnote-ref-2)