**Disease card**

**WITHERING SYNDROME OF ABALONE**

## Pathogen information

### 1. causative agent

1.1. pathogen type: bacterium

1.2. disease name and synonyms: abalone rickettsiosis, withering syndrome of abalone

1.3. pathogen common name and synonyms: abalone rickettsia.

1.4. taxonomic affiliation

1.4.1. pathogen scientific name: *Candidatus Xenohaliotis californiensis*.

1.4.2. phylum, class, family etc…: bacterium, family Rickettsiaceae.

1.5. description of the pathogen: intracellular bacterium of the family Rickettsiaceae and is closely related to members of the genera *Erlichia, Anaplasma* and *Cowdria.*

1.6. authority: 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.

1.7. OIE status: Other Significant Diseases[1].

1.8. pathogen environment: brackish and marine waters

### 2. modes of transmission

2.1. routes of transmission: -

2.2. life cycle: incubation period of withering syndrome is prolonged and ranges between 3 and 7 months.

2.3. associated factors: the disease occurs at elevated water temperatures (~18°C and above).

2.4. additional comments: -

### 3. host range

3.1. host type: *Candidatus* Xenohaliotis californiensis infects members of the genus *Haliotis* including black abalone *(H. cracherodii)*, red abalone *(H. rufescens)*, pink abalone *(H. corrugata)*, green abalone *(H. fulgens)* and white abalone *(H. sorenseni)*.

3.2. host scientific names: *Haliotis cracherodii*, *H. rufescens*, *H. corrugata*, *H. fulgens* and *H. sorenseni*.

3.3. other known or suspected hosts: the bacterium is known to cause disease in black, red, pink and green abalone.

3.4. affected life stage: all stages.

3.5. additional comments: Susceptibility varies with species.

[1] http://www.oie.int/eng/normes/fcode/A_summry.htm

**4. geographic distribution**

4.1. region: *Candidatus* Xenohaliotis californiensis occurs along the south-west coast of North America.

4.2. country: in California, USA and Baja California, Mexico.

4.3. additional comments: the geographical range of the aetiological agent is suspected to be broad where California red abalones, *Haliotis rufescens*, are cultured ; as infected abalones have been transported to Chile, Japan, Israel and other countries.

**Disease information**

1. clinical signs and case description

1.1. host tissues and infected organs: *Candidatus* Xenohaliotis californiensis infects the gastrointestinal epithelial cells of the posterior oesophagus, digestive gland and, to a lesser extent, intestine. Severe infections result in withering syndrome. Decrease in feeding, depletion of glycogen reserves followed by use of the foot muscle as an energy source and death are also noted.

1.2. gross observations and macroscopic lesions: morphological changes in the digestive gland, which vary between species, may include degeneration (atrophy of tubules, increase in connective tissues and inflammation) and/or metaplasia of the digestive tubules. The metaplasia involves the replacement of terminal secretory/absorptive acini with absorptive/ transport ducts similar in appearance to the post-oesophagus. Some hyperplasia of the absorptive/ transport ducts may also be involved. The foot of affected individuals contains fewer and less organised muscle bundles, abundant connective tissue and may contain more cerous cells than unaffected individuals.

1.3. microscopic lesions and tissue abnormality: The dimorphic rod-to-spherical shaped bacterium measures an average of 332 x 1550 nm in the bacillus form and an average of 1405 nm in the spherical morphotype. The bacteria reproduce within intracytoplasmic vacuoles 14–56 μm in diameter.

2. social and economic significance: cumulative mortality has been recorded at over 99% in black abalones and over 30% in red abalone.

3. zoonotic importance: none known.

4. diagnostic methods

4.1. screening methods

4.1.1. level I: none.

4.1.2. level II: for histological examination, remove the shell and cut several 3–5 mm cross sections that contain posterior oesophagus, digestive gland, and foot muscle and placed in Davidson's AFA solution (see *Asian Diagnostic Guide*) for 24 hours and process for routine paraffin histology. Sections should be stained with haematoxylin and eosin and viewed by light microscopy for RLP (Rickettsiales-like prokaryote) inclusions in the post-oesophagus and digestive gland, and morphological changes in the digestive gland and foot. It is recommended that sections should be examined at ×200 or ×400 magnification. *Candidatus* Xenohaliotis californiensis may be morphologically similar to other marine rickettsial bacteria. Definitive diagnosis by histology must include the presence

of the bacterium and morphological changes to the digestive gland and may include those of the foot muscle.

4.1.3. level III: none

4.2. presumptive methods

4.2.1. level I: no specific signs. Decrease in feeding, depletion of glycogen reserves followed by use of the foot muscle as an energy source and death may be considered.

4.2.2. level II: cytological examination of tissue imprints may be used to detect moderate to high intensities of infection of *Candidatus* Xenohaliotis californiensis. However, histology is more sensitive than tissues imprints. For this purpose, excise a section of the post-oesophagus, mince and lay on a slide, dry with a hair dryer for ~20 minutes. Stain the slides using a fluorescent stain for nucleic acid such as propidium iodide or Hoechst. Incubate in the dark for 3 minutes and view by epifluorescence at ×200 magnification. Bacterial inclusions are differentiated from host nuclei by size and frequency. However, if the sample slides are to be retained for future examination, they should be thoroughly dried and stored desiccated until staining. Inclusions of the bacterium, 14–56 µm in diameter, appear interspersed with the smaller host nuclei. An observation time of 5 minutes per slide is sufficient at ×200 magnification.

4.2.3. level III: A positive polymerase chain reaction (PCR) amplification is only a presumptive diagnosis because it detects DNA and not necessarily a viable pathogen; other techniques, preferably histology and *in-situ* hybridisation, must be used to visualise the pathogen. The PCR primers developed for *Candidatus* Xenohaliotis californiensis detection target small subunit ribosomal DNA and have been shown to be sensitive and specific for this pathogen and specifically amplify a 160 base-pair segment. Primers are currently designated as: RA 5-1 (5'-GTT-GAA-CGT-GCC-TTC-AGT-TTA-C-3') and RA 3-6 (5'-ACT-TGG-ACT-CAT-TCA-AAA-GCG-GA-3'). PCR amplification is performed in a standard 50 µl reaction volume containing 10 mM Tris, pH 8.3 (at 25°C), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% (w/v) gelatin, 400 µM of dNTP, 5 µM tetramethyl ammonium chloride, 40 pmoles of each primers, 2 units of Taq polymerase, and template DNA. The programme for amplification reaction is: initial denaturation at 95°C for 5 minutes, 40 cycles at 95°C for 1 minute, 50°C for 30 seconds, and 72°C for 30 seconds, and a final extension at 72°C for 10 minutes.

4.3. confirmatory methods

4.3.1. level I: none

4.3.2. level II: where losses have been observed within the known geographical range of withering syndrome, visualisation of intracellular bacterial foci within digestive epithelia, by histological examination, may be considered to be a confirmatory method. However, confirmation by *in-situ* hybridisation is recommended to verify the identity of the rickettsial bacteria in abalone species previously not known to be susceptible to the bacterium or in a new geographical location.

4.3.3. level III:

4.3.3.1. *in-situ* hybridisation is the method of choice for confirming identification because it allows visualisation of a specific probe hybridised to the target

organism. *In-situ* hybridisation has recently been developed to detect Rickettsiales-like prokaryotes in tissue sections. Specific labelled oligonucleotide probes hybridise with the small subunit ribosomal RNA of the bacterium. Positive and negative controls must be included in the procedure. The sequences of the probes designated as RA 5-1, RA 3-6, RA 3-8 and RA 5-6 are, respectively: 5'-GTT-GAA-CGT-GCC-TTC-AGT-TTA-C-3', 5'-ACT-TGG-ACT-CAT-TCA-AAA-GCG-GA-3', 5'-CCA-CTG-TGA-GTG-GTT-ATC-TCC-TG-3', and 5'-GAA-GCA-ATA-TTG-TGA-GAT-AAA-GCA-3'. Reaction of hybridisation is performed overnight at 40°C.

4.3.3.2. transmission electron microscopy examination may also be use; rod-shaped, ribosome-rich prokaryotes with trilaminar cell walls accumulated into intracellular colonies within membrane-bound vacuoles in the cytoplasm of gastrointestinal epithelial cells are observed

5. control methods: Reducing densities and application of an oxytetracycline-medicated diet may reduce losses.

**Selected references**


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