Infection with *Bonamia ostreae*

AETIOLOGY

CLASSIFICATION OF THE CAUSATIVE AGENT

Bonamia ostreae is a protistan parasite of the phylum Haplosporidia.

Infection with *Bonamia ostreae* is also known as bonamiosis, microcell disease, haemocyte disease of flat oysters.

RESISTANCE TO PHYSICAL AND CHEMICAL ACTION

Currently unknown.

EPIDEMIOLOGY

HOSTS

- *Bonamia ostreae* naturally occurs in *Ostrea edulis* and when moved in endemic zones in *O. puelchana, O. angasi, O. denselamellosa, Ostrea chilensis (= Tiostrea chilensis = Tiostrea lutaria),* and probably *Crassostrea ariakensis (= Crassostrea rivularis*).

- Other oyster species are suspected to be susceptible to *Bonamia ostreae* like *Ostrea conchaphila* (=*Ostrea lurida*).

TRANSMISSION

- Although the life cycle outside the host is unknown, it has been possible to transmit the disease experimentally in the laboratory by cohabitation or inoculation of purified parasites.

OCCURRENCE

The geographical distribution of *Bonamia ostreae* is Europe (France, Ireland, Italy, The Netherlands, Spain and the United Kingdom excluding Scotland), The United States of America (California, Maine and Washington States) and Canada (British Columbia).

The parasite may occur throughout the year but prevalence and intensity of infection tend to increase during the warm season. There is a seasonal variation in infection by *B. ostreae* with the highest prevalence occurring in September. A prepatent period of at least 3 months is observed.

For detailed information on occurrence, see recent issues of *World Animal Health* and the OIE Web site.

DIAGNOSIS

CLINICAL DIAGNOSIS

- There are no pathognomonic signs of bonamiosis.

LESIONS

- Bonamiosis is a lethal infection of the haemocytes of flat oysters, sometimes accompanied by yellow discoloration and extensive lesions on the gills and mantle. However, most of the infected oysters appear normal. Histologically, lesions occur in the connective tissue of the gills, mantle, and digestive gland. This intrahaemocytic protozoan quickly becomes systemic with overwhelming numbers of parasites coinciding with the death of the oysters.

DIFFERENTIAL DIAGNOSIS

- If detected in other host species than *Ostrea edulis* or outside the known range of *Bonamia* ostreae, electron microscopy and molecular tools like PCR-RFLP must be used to identify and distinguish the detected organism from other microcell species *(Bonamia exitiosa*, *B. roughleyi* and *Mikrocytos mackini)*.

LABORATORY DIAGNOSIS

Procedures

- Histology or tissue imprints are routine techniques used for surveillance. When mortalities occur, PCR can be used in addition to histocytological techniques. When a pathogen is detected, PCR or *in situ* hybridization should be used to assess at least the parasite phylum and electron microscopy and PCR-RFLP to determine the species identification.

- For details refer to OIE *Diagnostic Manual for Aquatic Animal Diseases* (the *Manual*).

Histology

- Samples are handled in accordance with classical histological methods. Sections are stained for example with haematoxylin–eosin. It is recommended that two sections per oyster be examined.

- The parasite (2–5 µm in size) occurs within the haemocytes or extracellularly. However, the parasite has to be observed inside the haemocytes for a positive diagnosis to be made.

Cytological examination: tissue imprints

- Make an impression of oyster heart or gill tissue on a glass histological slide, air-dry and fix in methanol or in absolute ethanol. The prepared imprints are

stained using a commercially available blood staining kit, in accordance with the manufacturer's instructions.

- The parasite (2–5 µm in size) has basophilic cytoplasm and an eosinophilic nucleus (colours may vary with stain used). It may be observed inside or outside the haemocytes. The organisms are enlarged by this method compared with those observed in histology.

Electron microscopy examination

- Classical transmission electron microscopy procedures used for molluscs are given in the *Manual*.

- Differences exist between *Bonamia ostreae* and *Bonamia exitiosa* forms: number of haplosporosomes and lipid droplets, morphology of mitochondria, and nucleus and cytoplasm ratio.

Molecular techniques: Polymerase Chain Reaction (PCR) and *in situ* hybridization (ISH)

- PCR and ISH have been recently introduced for the detection of *Bonamia ostreae*.

- The small subunit ribosomal RNA gene was sequenced and specific primers and probes were designed. These techniques require some training and specific equipment. However, as PCR and *in situ* hybridization can solve difficulties linked to low infestation level and low specificity of histocytological methods, these techniques could be adopted by many laboratories in the next few years.

- *Bonamia ostreae* can be distinguished from *B. exitiosa* and *B. roughleyi* by restriction fragment length polymorphism (RFLP) analysis, by digesting PCR products with two restriction enzymes.

PREVENTION AND CONTROL

- There is no applicable treatment for molluscs.

SANITARY PROPHYLAXIS

Free countries, zones and aquaculture establishments

- Targeted pathological surveillance for occurrences of any abnormal mortality outbreaks.

- Policy and procedures for importation of life molluscs.

REFERENCES

Chapter 3.1.1. in the OIE *Diagnostic Manual for Aquatic Animal Diseases*, OIE, Paris, France.

Chapter 3.1.1. in the OIE *International Aquatic Animal Health Code*, OIE, Paris, France.

Other Reference Experts and Laboratories in 2006	
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