

Diagnosis by histo-cytopathology of *Bonamia* spp. in flat oysters *Ostrea* spp.

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Editions

Edition	Date	Updated part
1	01/03/05	Creation
2	09/10/07	§ 2 (references), 5.2 & 5.3 (pictures)
3	19/08/08	Title, § 1, 2, 3, 5.2 & 5.3
4	22/04/09	Title, §1, 2 and 5
5	17/05/13	§ 1, 2 and 3

Diagnosis by histo-cytopathology of *Bonamia* spp. in flat oysters *Ostrea* spp.

1. Scope

This procedure explains the diagnostic tests used for Haplosporidian *Bonamia* spp. presumptive diagnosis in the flat oysters *Ostrea* spp. following histological or cytological processing of samples.

2. References

- [Council Directive](#) 2006/88/EC of 24 October 2006 on "animal health requirements for aquaculture animals and products thereof, and on the prevention and control of certain diseases in aquatic animals".
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- Pichot Y., Comps M., Tige G., Grizel H. et Rabouin M.A. (1979) Research on *Bonamia ostreae* gen. n., sp. n., a new parasite of the flat oyster *Ostrea edulis* L. *Rev. Trav. Inst. Peches Marit.* 43:131-140

3. General information

Bonamiosis is a lethal infection of the haemocytes of flat oysters (*Ostrea* spp.) caused by the haplosporidians *Bonamia* spp. It is sometimes accompanied by yellow discoloration and extensive lesions on the gills and mantle. However most of the infected oysters appear normal. Lesions occur in the connective tissue of the gills, mantle, and digestive gland. These intrahaemocytic protistans quickly become systemic often leading to the death of the oysters. Bonamiosis can be transmitted experimentally by cohabitation or inoculation. *Bonamia ostreae* and *B. exitiosa* have been observed in the oyster *Ostrea edulis* of Europe. Several *Bonamia* species have been observed in the world: *B. exitiosa* in *Ostrea chilensis*, *O. angasi*, *O. edulis* and *O. stentina*, *B. perspora* in *Ostreola equestris*, *B. roughleyi* in *Saccostrea glomerata*, and *Bonamia* sp. in *Ostrea puelchana*. Experimental assays have indicated a low infectivity of *B. ostreae* to *Crassostrea ariakensis* (Audemard et al. 2005).

Bonamia ostreae may occur throughout the year, but the highest prevalence is usually observed in late winter-early spring. *Bonamia ostreae* has been reported from France, Ireland, Italy, the Netherlands, Spain, the United Kingdom, Norway, Morocco, Canada and the U.S.A. (California, Maine, Washington State). *B. exitiosa* has been observed in flat oysters *Ostrea edulis* from Spain (Galicia), Italy (Adriatic coast), France (Mediterranean coast) and England (South-West coast).

Examination of stained tissue sections or tissue imprints and PCR and Real Time PCR of susceptible organs are the OIE recommended methods for screening and presumptive diagnostic. Transmission electron microscopy can be used as confirmatory diagnostic method. Differentiation between *Bonamia* species can be achieved 1) by using a PCR-RFLP technique on positive PCR products for *Bonamia* spp. detection and 2) by sequencing.

Other information is available on the [EURL website](#).

4. Equipment and environmental conditions

Binocular microscope for histological and cytological examination, equipped with different normal dry objectives (10X, 20X, 40X and/or 60X) and a 100X (oil) objective with immersion oil. Microscope should be adjusted with [Koehler](#) illumination technique.

5. Operating procedure

5.1. Imprints examination

Slides with tissue imprints (heart, gill, oyster larvae) must be observed first with 10X or 20X dry objectives: look for areas containing numerous haemocytes per field at 20X objective.

Bonamia spp. cells occur within or outside the haemocytes (Figure 1). They appear as very small cells (2-5 µm wide) with a basophilic cytoplasm and an eosinophilic nucleus. Because they spread on the slide, they can look wider than in histology examination. Multinucleated cells can be observed (Figure 2).

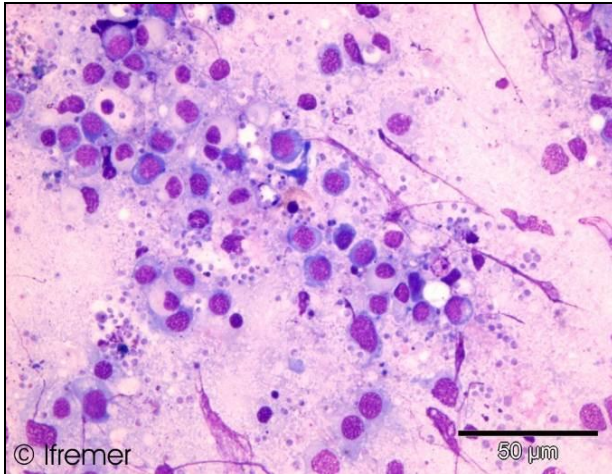


Figure 1: *Ostrea edulis* heart imprint observed with X40 objective. Hemacolor® staining.

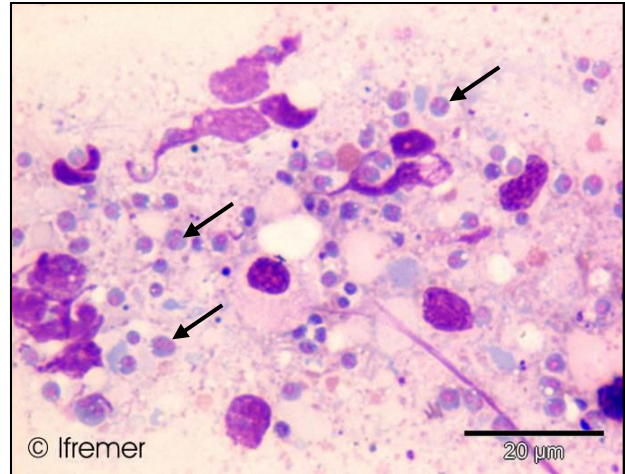


Figure 2: Heart imprint with some binucleated *Bonamia ostreae* cells (arrows). Hemacolor® staining (X100 objective).

5.2. Histological examination

Bonamia spp. can be observed within the haemocytes or freely in connective tissue or sinuses of gill, gut and mantle epithelium, often associated with intense inflammatory reaction (Figure 3). The parasites appear as very small cells of 2-5 µm wide (Figure 4). *B. exitiosa* usually appears larger than *B. ostreae* with a more centred nucleus. To avoid any doubt the parasite has to be observed inside the haemocyte for a positive diagnosis. This is to avoid false positive results.

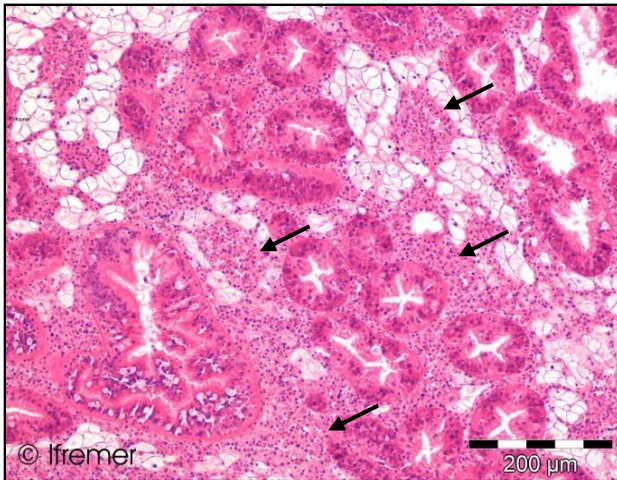


Figure 3: Haemocytes infiltration within connective tissue of digestive gland and mantle heavily infected by *Bonamia ostreae*. H & E staining (X10 objective).

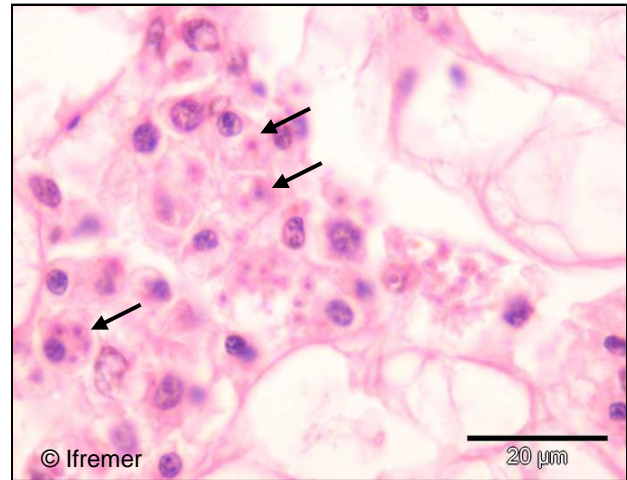


Figure 4: *Bonamia exitiosa* (arrows) within haemocytes in connective tissue of an *Ostrea edulis* oyster from Galicia, Spain (slide courtesy of A. Villalba). H & E staining (X100 objective).

5.3. Results statement

Results are expressed **qualitatively** (infected = **positive** / non-infected = **negative**) at the genus level (i.e. *Bonamia* sp.). They can also be expressed according to **semi-quantitative** scales for histology and imprints (low, medium, heavy infection).

Grading system for the severity of *Bonamia* sp. infections in *Ostrea edulis* when observed by histology (proposal from Steve Feist, CEFAS, UK) :

- Grade 0 – No infection present in all the section
- Grade 1 – Few (1 to 5) small focal areas of haemocyte proliferation, one or more of which contain few to moderate numbers of intracellular parasites.
- Grade 2 – Multifocal (5 to 10) or zonal areas of haemocyte proliferation occupying gill tissue, digestive gland/gonad or both. Parasites will be more abundant than in Grade 1 and some may be extracellular in vessels or connective tissues.
- Grade 3 – Diffuse infections with large numbers of haemocytes infected with one to several parasites present in each infected cell. Generalised haemocyte proliferation may or may not be present. Disrupted or necrotic host tissues will be evident.