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Bonamiosis of oysters caused by *Bonamia exitiosa*

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Contents

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Susceptible species

Various oysters, primarily in the genus *Ostrea* (*O. chilensis*, *O. angasi*, *O. edulis*, *O. stentina, O. auporia*, *O. equestris*, *O. puelchana*, *O. lurida*) but also *Crassostrea ariakensis* and *C. virginica* and probably *Saccostrea glomerata* (Dinamani *et al.,* 1987; Burreson *et al*., 2004; Carnegie *et al*., 2006; Corbeil *et al*., 2006; Abollo *et al.,* 2008; ICES, 2013; Carnegie *et al*., 2014; Hill *et al*., 2014).

Disease name

Bonamiosis

Aetiological agent

Bonamia exitiosa, Phylum Haplosporidia (Carnegie *et al*., 2000), a parasite of oyster hemocytes. Transmission is presumed to be direct.

Geographical distribution

Bonamia exitiosa was originally described from *Ostrea chilensis* in New Zealand (Dinamani *et al*., 1987; Hine *et al*., 2001). Since 2003, the parasite has been observed on both Atlantic and Pacific coasts of the USA, detected south of Cape Hatteras in the east (Burreson *et al*., 2004; Dungan *et al*., 2012) but also in Massachusetts (ICES, 2014, and in California in the west (Hill *et al*., 2014); in southeastern Australia (Corbeil *et al*., 2006; Carnegie *et al*., 2014); along the Atlantic and Mediterranean coasts of Europe and North Africa, including Spain, France, the United Kingdom, Italy, Portugal and Tunisia (Abollo *et al*., 2008; Hill *et al*., 2010; Narcisi *et al*., 2010; ICES, 2010; Carrasco *et al*., 2012; Longshaw *et al*., 2013; Batista *et al*., 2016); and in Argentina (Kroeck and Montes, 2005).

Associated environmental conditions

Depending on the host and geographic location, clinical disease may be associated with temperatures ranging from below 10˚C (Cranfield, 1968; per Hine, 1991) to over 30˚C (Carnegie *et al*., 2008). The parasite is frequently observed year-round with only a modest annual prevalence cycle displayed, as in *O. chilensis* (Hine, 1991). In *C. ariakensis*, however, prevalence and clinical disease were found to be sharply higher in the warmer summer and early fall months (Carnegie *et al*., 2008). *B. exitiosa* displays a preference for euhaline habitats and may be inhibited by salinities below 30 ppt (Bishop *et al*., 2006; Audemard *et al*., 2008).

Significance

Bonamia exitiosa is acutely pathogenic in some hosts, including *O. chilensis*, *O. puelchana*, and *C. ariakensis* (Dinamani *et al*., 1987; Burreson *et al*., 2004; Kroeck and Montes, 2005). It can infect these species at high prevalences and intensities, causing significant mortality. From what has been reported, *B. exitiosa* is somewhat less pathogenic in other

host species. Unambiguous histological evidence of infection of *S. glomerata* from Australia and *O. auporia* from New Zealand is lacking although *B. exitiosa* DNA has been sequenced from both hosts (Carnegie *et al*., 2014; Hill *et al*., 2014) and *O. auporia* (as well as *O. stentina*) has been proposed to be synonymous with *O. equestris* (Shilts *et al*., 2007), which is clearly susceptible. Effects on *C. ariakensis* and *C. virginica* have been focused primarily on young (< 1 year old) seed, with observations exclusively limited to an aquaculture context (Burreson *et al*., 2004; Bishop *et al*., 2006; ICES, 2013). Because it is regarded as a significant pathogen, *B. exitiosa* looms as an impediment to fisheries and aquaculture commerce even where it is not acutely pathogenic, as for *O. edulis* in Europe and *C. virginica* in the USA. The significance of new observations is uncertain. The discovery of *B. exitiosa* in *O. edulis* in Europe, for example, may represent improved resolution of parasite diversity (*Bonamia ostreae* also being present) through the application of molecular diagnostics and DNA sequencing. The recent observation in *C. virginica* in North Carolina, USA may represent a temporary host switch under relatively stressful conditions of aquaculture, the parasite never having been observed in *C. virginica* from the region but known to infect *O. equestris* locally. Infection of *C. virginica* in Massachusetts, USA, remote from documented populations of both *B. exitiosa* and *O. equestris*, defies easy explanation.

Gross clinical signs

Bonamiosis caused by *B. exitiosa* cannot be diagnosed based on gross signs.

Control measures and legislation

Methods for control of *B. exitiosa* are not well established. Care should be taken to avoid introduction of the parasite to *B. exitiosa*-free areas. The parasite may potentially be avoided through selection of culture sites in waters of intermediate salinity (< 25, Audemard *et al*., 2008) unfavourable to it, although this strategy is not practical for more stenohaline *Ostrea* species like *O. edulis*. The effectiveness and practicality of low-salinity treatment of infected oysters remains to be determined. Infection with *B. exitiosa* is a World Organisation for Animal Health (OIE)-listed disease.

Diagnostic methods

The small size of *B. exitiosa* cells (2–4 μm) makes microscopic detection challenging in cases where infection intensity is light, generally necessitating a dual strategy of detection by both microscopic and molecular means. Microscopically, *B. exitiosa* cells can be recognized in both standard histological preparations as well as stained heart or haemolymph smears as primarily uninucleate forms inhabiting the cytoplasm of oyster haemocytes and (to a lesser extent) free in oyster haemolymph. Polymerase chain reaction (PCR) assays specific for *B. exitiosa* have been developed (Carnegie *et al*., 2008; Ramilo *et al*., 2013), the latter adaptable for use in a SYBR Green real-time PCR format or multiplexed in conventional PCR format with an assay presented in the same publication for *B. ostreae*. An older conventional assay detecting *B. exitiosa* via PCR-restriction fragment length polymorphism (RFLP, Hine *et al*., 2001) remains in wide use. Available *in situ* hybridisation (ISH) assays (Cochennec *et al*., 2000; Carnegie *et al*., 2003) remain genus-specific at best. Transmission electron microscopy (TEM) is not a practical tool for *B. exitiosa* diagnosis.

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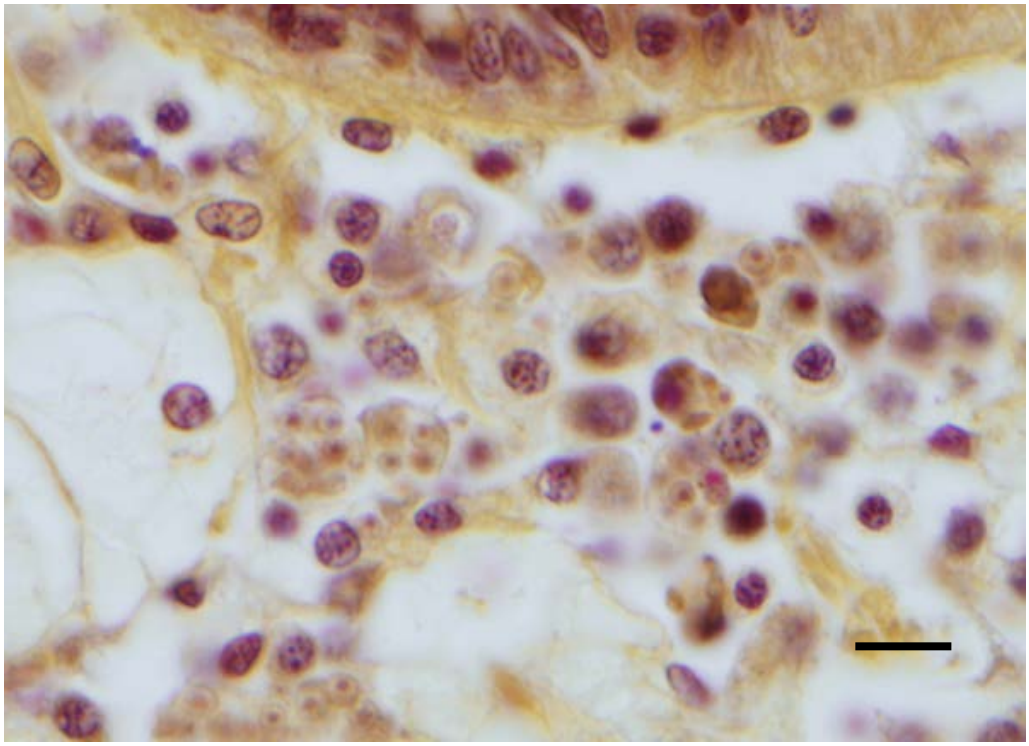
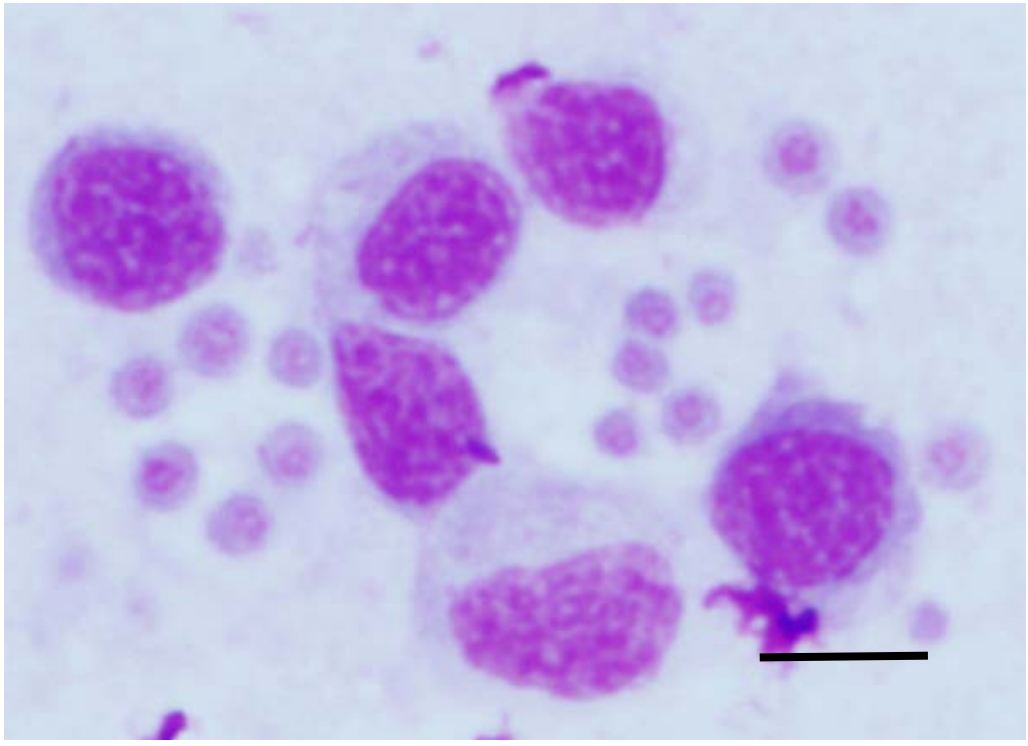


Histological section of phloxine-tartrazine-stained tissue from a *Bonamia exitiosa*-infected oyster (*Crassostrea ariakensis*) showing parasite cells both intrahaemocytic and free in haemal spaces of connective tissues. Bar = 10 microns. (Photo: R. B. Carnegie, VA Institute of Marine Science. Histology courtesy of C. F. Dungan, Maryland Department of Marine Resources, USA).



Cytological preparation of Hemacolor (Merck)-stained oyster hemolymph from a *Bonamia exitiosa*-infected oyster (*Crassostrea ariakensis*) showing parasite cells distributed extracellularly among oyster haemocytes. Bar = 10 microns. (Photo: R. B. Carnegie, VA Institute of Marine Science).

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