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Marteiliosis of oysters caused by *Marteilia refringens*

Original by Michel Comps

Revised and updated by Tristan Renault and Susan E. Ford

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Susceptible species

Primarily the European flat oyster, *Ostrea edulis.* Other species of *Ostrea, O. puelchana, O. Angasi,* and *O. chilensis (= Tiostrea chilensis = Tiostrea lutaria)* can become infected when moved into enzootic zones. Also found in the mussels *Mytilus edulis* and *M. galloprovincialis,* the razor clam *Solens marginatus,* and the striped clam *Chamelea gallina.*

Disease name

Marteiliosis, Aber disease, Digestive gland disease

Aetiological agent

There has been considerable confusion concerning the taxonomic affinities and phylogenic placement of *Marteilia refringens*, the aetiological agent of marteiliosis in the European flat oyster. Although it was originally assigned to the phylum Paramyxea, a recent analysis has suggested that *M. refringens* is closely related to parasites in the phylum Haplosporidia.

Direct transmission of the parasite between oysters has not been demonstrated. Several organisms have been implicated as a potential intermediary host of *M. refringens*: copepods such as *Acartia grani*, *Canuella perpleja* and *Euterpina acutrifons*, the cnidarian *Cereus pedunculatus*, decapod larvae, and the fish *Pomatochistus microps,* suggesting the involvement of zooplankters in the life cycle and as possible intermediate hosts. With the exception of *A. grani*, however, the presence of *M. refringens* in these zooplankters has been inferred by the finding of amplifiable DNA rather than histological observation. Experimental transmission of the pathogen from infected oysters to *A. grani* was successful; however there was no evidence of transmission from the copepod to the bivalve.

Geographical distribution

Marteilia refringens has been reported in Europe (from Greece to the Netherlands) and in North Africa (Morocco).

Associated environmental conditions

Development of *M. refringens* infections is directly related to high water temperature and low salinity. High prevalence of the disease is reported in the most enclosed farming areas. The parasite can survive outside the host from several days up to 2–3 weeks, depending on the environmental conditions.

Significance

Infection with *M. refringens* causes a lethal disease and has provoked mass mortality of the flat oyster, *O. edulis*, in Europe. Death occurs during the second year after

initial infection. Infection prevalence can reach 80–100%. In France, between 1980 and 1983, the conjunction of marteiliosis and bonamiosis outbreaks resulted in economic losses estimated at €240 million in direct dockside value, €200 million of added value, and a 20% loss of employment in the oyster industry.

Although mussels are known to be infected by *M. refringens* in Europe, they are usually not adversely affected by marteilioisis.

Gross clinical signs

Emaciation, discolouration of the digestive gland and cessation of growth have been observed among infected oysters; however, they are not specific to marteiliosis and the disease cannot be diagnosed on the basis of gross signs.

Control measures and legislation

The primary method of control is by restriction of the movement of infected animals to areas known or suspected to be free of *M. refringens*, which is listed as a notifiable pathogen by the EU legislation (2006/88/CE) and by the World Organisation for Animal Health (WOAH; – http://www.oie.int/animal-health-in-the-world/oie-listed-diseases-2012/). Although transfer regulations have been developed in order to avoid the introduction of animals from an enzootic area to areas considered to be free of *M. refringens*, marteiliosis may still become a problem because of the occurrence of *M. refringens* in mussels. Whether or not mussels play a role in the transmission of the parasite to oysters has yet to be established.

Planting and harvesting oysters outside the period when they are most likely to become infected with *M. refringens* can significantly reduce the risk of marteiliosis. Moreover, given the putative role of copepods in *M. refringens* transmission, mapping the geographical and temporal distribution of this possible intermediate host could provide information for developing a suitable avoidance strategy.

Diagnostic methods

Tissue imprints can be made using the digestive gland from live or gaping bivalves. *Marteilia refringens* appears as cells ranging in size up to 30–40 μm. Using Wrights, Wright-Giemsa or equivalent stains, parasites demonstrate a basophilic cytoplasm and an eosinophilic nucleus. Pale halos around large, strongly stained (refringent) granules are typical, and in larger cells, cell-within-cell arrangements are observed.

Histological sections should include digestive gland, gills, and palps and should be stained with hematoxylin and eosin or an equivalent stain. Different stages of the parasite can be observed in infected oysters and mussels. In tissue sections, *M. refringens* cell diameters range from 4 to 40 μm. Young plasmodia (uninucleate) are mainly found in the epithelium of labial palps and stomach. Sporulation involves divisions of cells within cells and takes place in the epithelium of the digestive tubules and ducts. Refringent granules (Figure 1) appear in the course of sporulation, but are not observed in early stages (Figure 2). In late phases of infection, sporangia are observed free in the lumen of the digestive tract.

A Polymerase chain reaction (PCR) protocol targeting the first internally transcribed spacer (ITS-1) of the rRNA region has been developed for the detection of *M. refringens*. No cross-reaction with parasites from other genera has occurred in tested samples. The DNA sequencing and specifically the analysis of the small subunit ribosomal genes (SSU rRNA) of *Marteilia* spp. purified from infected oysters (*O.*

edulis) and mussels (*M. edulis*) demonstrated identical sequences. Polymorphisms in the ITS-1 region of the rRNA gene allowed the identification of two *Marteilia* genetic types in European waters, named "O" and "M", which appeared to be linked to the host species, oyster and mussels, respectively. However, "O" types were found in mussels and "M" types were found in oysters, which suggests several cross-species transmissions of *Marteilia* between mussels and oysters, and also that the two identified *Marteilia* genetic groups are not strongly related to the host species.

An *in situ* hybridization protocol has been developed and is based on the use of Smart2, a 266 bp digoxygenin-labelled PCR-generated probe targeting the SSU rDNA. Smart2 is able to detect *Marteilia* species including *M. refringens* infecting flat oysters and mussels, and *M. sydneyi* (a parasite of the Sydney rock oyster, *Crassostrea glomerata)*. The values of specificity and sensitivity for *in situ* hybridization were estimated at 0.9 and 0.99, respectively, when co-validated with histology. *In situ* hybridization can help to detect early infections, which are difficult to find in traditional histological sections.

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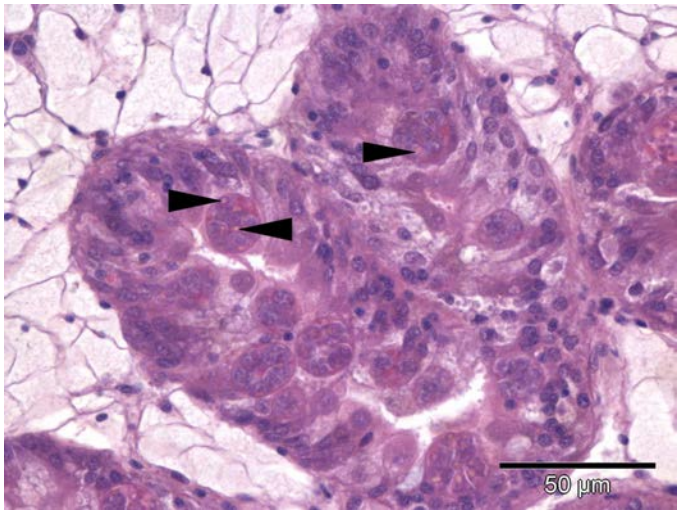

Figure 1. Digestive tubule epithelium of *Ostrea edulis* with *Marteilia refringens* sporangia (arrowheads). Tips of arrowheads point to refringent granules within the sporangia.

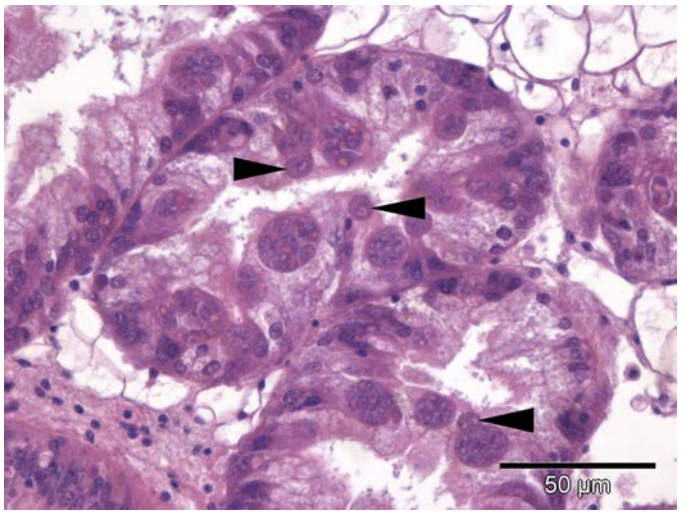

Figure 2. Young stages (without refringent granules) of *Marteilia refringens* (arrowheads) within the digestive epithelium of *Ostrea edulis*. H&E staining.

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