CHAPTER 3.1.5.

PERKINSOSIS
(*Perkinsus marinus and P. olseni/atlanticus*)

GENERAL INFORMATION

Perkinsosis here refers only to the diseases caused by Perkinsus marinus *and* P. olseni/atlanticus. *Other described species of* Perkinsus *include* P. chesapeaki *from* Mya arenaria (26), P. andrewsi *in* Macoma balthica (14), *both from the east coast of the United States of America (USA), and* P. qugwadi *from* Patinopecten yessoensis *from western Canada (5). These species are not considered at this time to cause diseases notifiable to the OIE. Other unidentified* Perkinsus spp. *infect many species of bivalves in tropical and subtropical waters (22). Until more is known about the identity, biology and pathology of these other* Perkinsus *spp., their presence in any bivalve should be regarded as potentially serious and the OIE Reference Laboratory should be consulted.*

Perkinsosis is an infection of marine molluscs caused by protistan parasites of the genus Perkinsus. *Recent investigations using molecular sequence data (21, 39, 42) indicate that* Perkinsus *is not in the phylum Apicomplexa as suggested by ultrastructural data (25), but is closely related to the Dinoflagellida. Some authors have placed* Perkinsus *in the phylum Perkinsozoa (33), or the phylum Dinozoa subphylum Protalveolata (12), but more molecular data are necessary on* Perkinsus *and genera related to* Perkinsus *before phylogenetic relationships will be clear.*

Perkinsus marinus *causes disease of economic importance in* Crassostrea virginica (2, 7). *Although infection of* C. gigas *and* C. ariakensis *is possible, these species seem to be more resistant to the disease (4, 9, 10).* Perkinsus marinus *was formerly named* Dermocystidium marinum *(29), and then* Labyrinthomyxa marina *(30). Infection by* P. marinus *is commonly known as 'Dermo disease' (19).* Perkinsus marinus *is found on the east coast of the USA (2, 7) from Maine to Florida, and along the Gulf of Mexico coast to the Yucutan Peninsula (6). The recent northward range extension of* P. marinus *into Delaware Bay, New Jersey, Cape Cod and Maine, USA, is attributed to repeated introductions over several years in conjunction with recent increases in winter sea-surface temperatures (13, 18). The effects* of P. marinus *infection in* C. virginica *range from a pale appearance of the digestive gland and reduction in condition index, to severe emaciation, gaping, retraction of the mantle, inhibition of gonadal development, retarded growth, and death (27, 28).*

Perkinsus olseni *was originally described from* Haliotis ruber *in Australia (24) and* P. atlanticus *was originally described from* Ruditapes decussatus *in Portugal (3). Similarities in the nucleotide sequences of the internal transcribed spacers (ITS) of the ribosomal RNA gene cluster (20) have long suggested that* P. olseni *is conspecific with* P. atlanticus. *Recently, the two species have been formally synonymised based also on sequence similarities in the rRNA nontranscribed spacer (NTS) region (32); Perkinsus olseni has taxonomic priority. Other susceptible hosts for* P. olseni/atlanticus *include* Haliotis cyclobates, H. scalaris, H. laevigata, Anadara trapezia, Austrovenus stutchburyi *and* Ruditapes philippinarum *(22, 23, 32, 34). The geographical distribution of* P. olseni/atlanticus *is eastern and southern Australia, New Zealand, Korea, Japan, Portugal, France, Italy and Spain (1, 3, 11, 22, 23, 34, 40).*

Proliferation of P. olseni/atlanticus *results in the disruption of connective tissue and epithelial cells leading to weakening. Cysts are visible macroscopically on the gills of infected* R. decussatus *(3).*

Abscesses occasionally can be noted in abalone (Haliotis *spp.*) *(24). Pustules up to 8 mm in diameter in the foot and mantle of infected* Haliotis *spp. reduce market value.* Perkinsus olseni/atlanticus *was directly associated with high losses of the abalone* H. laevigata *in Australia (22) and mortality in the clam* R. decussatus *in Portugal (3) and in the clam* R. philippinarum in *Korea (34).*

Morphology of life history stages is similar for all Perkinsus *species. Trophozoites, characterised by a large vacuole and a displaced nucleus, occur intercellularly in connective and epithelial tissue. Mature trophozoites divide by successive binary fission resulting in the release of 8–32 immature trophozoites (36, 44). The developmental cycle of* P. marinus *often occurs within phagocytes. Proliferation of all* Perkinsus *species is correlated with warm summer water temperatures (higher than 20°C) when pathogenicity and associated mortalities are highest. All life history stages appear to be infective (45). Under certain, poorly understood, conditions mature trophozoites enlarge and undergo zoosporulation. Although biflagellate zoospores are infective, the role of the zoospore in transmission in nature is unclear.*

Reference methods for the detection of Perkinsus *spp. are histological sections and culture in fluid thioglycollate medium (8, 17, 37, 38). For diagnosis, the recommended guidelines for sampling are those stated in Chapter I.2. of this* Manual.

EXAMINATION PROCEDURES

1. Screening Methods

1.1. Diagnosis by culture in Ray's fluid thioglycollate medium

Tissue samples measuring approximately 5 ´ 10 mm are excised giving preference to rectal, gill and mantle tissue from oysters and clams, and adductor or foot muscles or mantle for abalone, and placed in fluid thioglycollate medium (Difco) containing antibiotics. Recommended antifungal/antibiotics are 200 units of mycostatin (Nystatin), 500 units penicillin G and 500 mg dihydro-streptomycin per ml of media (37). Chloromycetin can be used in place of penicillin/ streptomycin (38). Incubation is at 22–25°C for between 4 and 7 days, in the dark.

Cultured parasites enlarge from 2–10 to 50–70 µm during incubation. After incubation, the fragments of tissue are collected and macerated with a scalpel blade on a glass slide, a drop of Lugol's iodine 1/5 solution is added, and the preparation is covered with a cover-slip and allowed to sit for 10 minutes. The preparations are examined in the fresh state. *Perkinsus* hypnospores are spherical and the walls stain blue or bluish-black with Lugol's iodine solution.

Infection intensity has been ranked (37) on a scale of 0 to 5 as follows: 0 = uninfected; 0.5 (very light) = fewer than ten parasites found in entire tissue preparation; 1 (light) = 11–100 cells present in entire preparation, parasites may be scattered or occur in isolated clusters of 10–15 cells; 2 (light moderate) = some areas free of parasites, but other areas show localised concentrations of 24–50 cells, or cells uniformly distributed so that 2–3 cells occur in each field at ´100 magnification; 3 (moderate) = parasites so numerous expect to find >3 cells in all fields at ´100 magnification, masses of >50 cells are still more or less localised; tissue does not show blue/black colour macroscopically; 4 (moderate heavy) = parasite cells present in large numbers in all tissues, but less than half of tissue shows a blue/black colour macroscopically; 5 (heavy) = parasite cells occur in enormous numbers; major part of tissue appears blue/black macroscopically.

1.2. Histology

General histological procedures are detailed in Chapter I.2. of this *Manual.* Cut a transverse section through the visceral mass that includes gill, mantle and digestive gland and place the tissue sample in a fixative, such as Davidson's fluid. The ratio must be no more than 1 volume of tissue to 10 volumes of fixative.

Samples are processed by conventional histological procedures. *Perkinsus* spp. are revealed by many nonspecific stains, such as haematoxylin & eosin. Histology is not as sensitive a technique as fluid thioglycollate culture and misses many light infections (19).

Perkinsus marinus infection is usually systemic, although cells can be localised in the gut epithelium. For *P. olseni/atlanticus*, infection is usually located in connective tissues. Thus, the connective tissue of all organs may harbour immature trophozoites, mature trophozoites ('signet-ring' stages), and tomont division stages containing 2, 4, 8, 16 or 32 developing trophozoites (19, 36, 44). Mature trophozoites are characterised by the presence of a vacuole that displaces the nucleus towards the periphery of the cell.

Morphology of all life history stages is similar for all species. The size of trophozoites in infected hosts varies from 2–10 µm for *P. marinus* up to 16 µm for *P. olseni/atlanticus*. However, morphological characters are similar for all *Perkinsus* species and size is highly variable, so morphology alone cannot be used to distinguish species.

2. Presumptive Diagnostic Methods

2.1. Diagnosis by culture in thioglycollate medium and histology

These methods, as described above (see Sections 1.1. and 1.2. of this Chapter), and used together, may be used as a presumptive diagnostic method for the genus *Perkinsus*. The techniques cannot be used to distinguish species.

2.2. Transmission electron microscopy

Transmission electron microscopy procedures are given in detail in Chapter I.2 of this *Manual*. The ultrastructure of the zoospore has been thought to be diagnostic for the genus *Perkinsus* (3, 5, 36). However, recent evidence based on molecular data has shown that *Colpodella*, a predatory flagellate that has identical ultrastructure to *Perkinsus* zoospores, is not closely related to *Perkinsus* (43). Thus, ultrastructural morphology of zoospores does not appear to be a suitable diagnostic tool even for the genus *Perkinsus*.

2.3. Polymerase chain reaction

A positive polymerase chain reaction (PCR) amplification is only a presumptive diagnosis because it detects DNA and not necessarily a viable pathogen. PCR primers have been developed for *P. marinus* (31, 41, 46) and *P. atlanticus* (15, 40), but they have not been thoroughly tested for inclusivity, specificity and sensitivity, especially in light of known sequence variability within a single *Perkinsus* species in the internal transcribed spacers (ITS) region (11, 16). A multiplex PCR (35) has been developed for *P. marinus*, *Haplosporidium nelsoni* (MSX) and *Haplosporidium costale* (SSO), but it has not been validated. Development of species specific and sensitive molecular diagnostic tools is an area of active research; however, PCR is not recommended at this time as a presumptive diagnostic method for any species of *Perkinsus*.

3. Confirmatory Identification of the Pathogen

3.1. ITS region sequence analysis

At the present time, the only way to confirm the identification of *Perkinsus* species is to compare ITS region nucleotide sequences with reference sequences deposited in the GenBank database (**HTTP://WWW.NCBI.NLM.NIH.GOV/ENTREZ/**). PCR primers have been designed that amplify the ITS region of any described *Perkinsus* spp. except *P. qugwadi*, from infected host tissue (11). The PCR products can then be sequenced for species identification.

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