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MAJOR PARASITIC AND PATHOLOGICAL CONDITIONS

Bonamiasis: A Model Study of Diseases in Marine Molluscs

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Abstract.—Research on bonamiasis, an epizootic disease of the edible oyster *Ostrea edulis* caused by the protozoan *Bonamia ostreae*, is discussed in relation to oyster farming, research technology, epizootiology, and management. Morphological and infectious characteristics of the parasite are described. Recent progress in isolation and purification of the parasite have permitted investigations into host defense mechanisms, parasite infectivity, and the development of a mollusc–pathogen model.

Since 1955, several important infectious diseases have been observed in molluscs, but, with few exceptions, no effective measures have been developed for eliminating them. This deficiency is a consequence of (1) a lack of knowledge concerning the host's anatomy, biology, and physiology; the infectious agent's metabolism, life cycle, etc.; and the mechanisms that control the host–parasite interrelationships; (2) the lack of a global view from which to study a disease; (3) a lack of coordination among research teams working in complementary disciplines; (4) insufficient contact and flow of information between research institutions, growers, and government agencies; and (5) a lack of training to recognize factors that induce or favor development of pathogenic agents.

The economic impact of the disease caused by *Bonamia ostreae*, a parasite of the edible oyster *Ostrea edulis* (Grizel 1983; Meuriot and Grizel 1984), led to research studies that have yielded original and substantial advances in mollusc pathology.

The Pathogen: Morphology, Ultrastructure, and Life Cycle

The tinctorial affinities of the cytoplasm of *Bonamia ostreae* reveal two different and distinct cellular types. Highly basophilic cells are seen most frequently. They are spheroid in shape, measure 2–3 μm in diameter, and contain a nucleus surrounded by a pale halo. Slightly basophilic cells are seen less frequently. They are somewhat elongated and measure 3–5 μm. In addition to these two types, binucleated cells and certain multinucleate plasmodial forms can also be observed, especially in postmortem oysters (Brehelin et al. 1982). The plasmodia can be as large as 6 μm in diameter and contain from 3 to 5 nuclei. Identical forms have also been reported by Dinamani et al. (1987) in *Bonamia* sp., a parasite of the New Zealand oyster *Tiostrea lutaria*. These different forms of *Bonamia ostreae* have been described from observations made with the electron microscope (Pichot et al. 1980; Brehelin et al. 1982; Comps 1983; Grizel 1985).

The cytoplasm of the highly basophilic cells contain many ribosomes 2.5 nm in diameter and mitochondria as large as 1.8 μm. In addition, the cells also contain two spheroid electron-dense inclusions and other dense particles that have the same structure as the haplosporosomes described by Perkins (1971) in several genera of the Ascetospora. The nucleus is 1 μm in diameter and contains a finely granular and electron-dense nucleoplasm. The cytoplasm of the slightly basophilic cells contains mitochondria with numerous tubular cristae, Golgi bodies composed of stacked saccules and small vesicles, and haplosporosomes. Spheroid inclusions are also present. The nucleus consists of a granular nucleoplasm and contains a dense nucleolus located peripherally. The cytoplasm of the plasmodial forms is similar to that of the highly basophilic cells.

The developmental cycle of *Bonamia ostreae* is unknown. Schizogony occurs in the host, but these divisions are probably rapid and are rarely observed. The same is likely true of the final multiplication of the parasite in the postmortem phase from which the plasmodial forms have been

described. Perkins (1988, this volume) discusses the probability that *B. ostreae* is one of the Haplosporidia.

The Disease

Gross Pathology

The most distinctive gross symptoms of bonamiasis are nonspecific gill lesions on one or more lamellae. According to Tigé et al. (1980), these lesions appear as perforations and indentations surrounded by characteristic yellowish bands. The principal microscopic findings are (1) dense cellular accumulations due to hemocytic infiltration and (2) *B. ostreae* present in the hemocytes or in the extracellular tissue spaces of the host oyster.

Descriptive Epidemiology

B. ostreae was discovered in Europe in edible oysters raised on Tudy Island (Brittany, France) in beds affected by high rates of mortality (Comps et al. 1980). This protozoan, previously undetected in molluscs cultivated along the French coast, was soon found in most areas of Brittany where the edible oyster was cultivated (Tigé et al. 1980). Only a few natural breeding areas off the coast of Brittany remain unaffected by the disease. Areas in which there was no breeding of edible oysters (such as Vendée) remain free of disease, as has the Mediterranean coast even after several transfers of infected oysters. The prevalence of the parasitic infection varies from one site to another and also from one age-class of edible oyster to another (Grizel 1985). This parasite has been found in several other European countries, including Spain, The Netherlands, England, and Ireland. Most likely, the parasite was introduced into France following the importation of spat from west coast hatcheries of the USA. The identity of *B. ostreae* and "microcells" imported from California in the 1960s (Katkansky et al. 1969) has been proposed on evidence from electron microscopy (Elston et al. 1986). This diagnosis has been confirmed with monoclonal antibodies specific for *B. ostreae* (E. Mialhe, unpublished data).

Analytic Epidemiology

Determination of the period of infection.—Experiments carried out in situ revealed that the disease is transmissible throughout the year (Tigé and Grizel 1984). Tigé and Grizel (1984) observed that different groups of presumably healthy edible oysters consistently became infected with the parasite 3–4 months after they were immersed in infested waters. The highest infection rates occurred during the summer.

Influence of environmental factors.—Temperature is not a major factor affecting transmission of the disease. In contrast to *Marteilia refringens*, which requires at least 17°C, infections of *B. ostreae* can be obtained at ambient temperatures as low as 4–5°C. The specific influence of salinity on infectivity has not been tested. However, infections have been observed in tanks where the salinity was 39‰. In general, there is insufficient evidence to describe the role of the environment in the expression of the disease or transmission of the parasite. Environment may have played a role in cases where the disease could not be transmitted in the laboratory.

Host resistance.—Susceptibility to *B. ostreae* varies with host genus and species. Tests conducted with several genera of molluscs living in a *Bonamia*-contaminated habitat did not result in infections (*Crassostrea gigas, Mytilus edulis, Ruditapes decussatus, R. philippinarum,* and *Cardium edule*). In New Zealand, however, *Bonamia* sp. has been detected in the genus *Haliotis* by M. Hine (Fisheries Research Center, Wellington, personal communication). Furthermore, Grizel et al. (1983) and Bougrier et al. (1986) observed during acclimatization tests that *Ostrea chilensis* and *Ostrea angasi* were susceptible to *B. ostreae*. Tests of resistance conducted with edible oysters collected from natural environments or hatcheries in different geographical areas revealed that all tested stocks contracted the disease.

Experimental Pathogenesis

Studies of experimental pathogenesis depend on the standardized purification and production of large quantities of the pathogenic agent. Recent progress made in the purification of certain molluscan protozoa (Mialhe et al. 1985) has enabled us to undertake such research.

In Vivo Pathogenesis

Poder et al. (1982) and Grizel (1985) conducted tests of pathogenicity by using either the proximity method or the method of inoculating infected hemocytes, but they did not quantify the parasite dose in either case. The second method resulted in a more rapid and uniform onset of the disease than the first. The time it takes for the infection to become evident varies with the method (Bachère et al. 1982; Poder et al. 1982) and can range from 15 d to 4 months. However, the rate of infection never exceeded 50% during the first 4 months of any experiment. Recently, experimental infections were initiated by injecting solutions of 50 μL

of seawater containing 1, 100, 10,000, or 1,000,000 *B. ostreae*-infected cells. The percentages of successful infections increased as the concentration of the inoculum was increased, 27% for 100 injected cells to 70% for 10^5 cells. Tests replicated five times with 30 injected edible oysters per concentration yielded similar results (Mialhe and others, unpublished data). At the lowest concentration, only one case of infection was detected in 150 edible oysters that were examined. These results showed the limits of diagnostic sensitivity and the need to increase the observation time to obtain the maximum response. The results also suggested high variability in individual responses. Improvement of the experimental technique should result in a useful and repeatable experimental model of parasitism in a marine mollusc.

In Vitro Pathogenesis

The availability of techniques to purify *B. ostreae* and the ability to maintain primary in vitro cultures of oyster cells, especially hemocytes, make it possible to apply the long-established techniques of in vitro mammal–parasite systems. Such research could characterize the mechanisms of cellular recognition, penetration of the host cell by the parasite, and parasite survival in the host cell. Fisher (1988) found that *B. ostreae* adhered more to *Crassostrea gigas* hemocytes than to *O. edulis* hemocytes in vitro, but latex beads adhered equally to both kinds of hemocytes. He suggested that hemocyte recognition of the parasite may play a role in the different susceptibilities to *B. ostreae* of these two oyster species. In our laboratory, we introduced *B. ostreae* into test vials containing hemocytes from *O. edulis* and *C. gigas*. One-half hour later, the parasites had adhered to the host cells, and after 2 h, *B. ostreae* entered the blood cells of both species of oysters, infecting the hyalinocytes as well as the granulocytes. In the latter cell type, the parasite was always enclosed in a parasitophorous vacuole (D. Chagot, unpublished data). Additional experiments are being conducted to answer other questions, such as (1) Is there simple cellular adhesion or a true process of recognition and binding? (2) Does *B. ostreae* actively enter the host cell, or is it phagocytized? (3) If phagocytized, how does *B. ostreae* survive in the phagocyte of *O. edulis?* (4) What happens to the parasite in the hemocyte of *C. gigas,* in which neither natural nor experimental infections have been observed? These important fundamental studies are intermediate steps that will lead to new paths of research to be pursued in genetics and therapy. Such research should broaden our present means to combat epizootic bonamiasis in marine molluscs.

Prophylaxis

After the discovery of *B. ostreae* in Europe, preventive measures were put into practice. These measures included destruction of the infectious areas by harvesting the edible oysters from them and prohibition of transfers of edible oysters from contaminated to disease-free areas. Van Banning (1987) studied eradication of *B. ostreae* during 1982–1986. He introduced test lots into previously contaminated areas and monitored them for 1 year. Until 1985, he detected the parasite at low levels (5%). The important question now is whether a large-scale reintroduction of edible oysters will again result in epizootic disease.

In France, the application of these disease control measures, together with health management practices, has made it possible to continue deepwater breeding of the edible oyster in the bays of Mont St. Michel (Cancale) and St. Brieuc (Binic) (Grizel et al. 1987). Excellent results have been obtained by breeding and growout to market size at the same site and by reducing spat density from 5 to 2 tonnes per hectare. Furthermore, data acquired in the course of epizootiological studies have been useful in developing a plan for safeguarding the edible oyster (Grizel et al. 1987). The absence of infection in juveniles made it possible to continue gathering spat even in infested bays.

Tests of disease resistance are being conducted on edible oysters that have shown individual resistance within a breeding population (Elston et al. 1987) or after experimental inoculations. We expect other experiments conducted in our laboratory to provide information about the genetic nature and transmissibility of this resistance.

Lastly, we have developed an immunodiagnostic procedure based on monoclonal antibodies and an enzyme substrate reaction. This procedure is more specific and sensitive than the commonly practiced histological techniques and provides a rapid diagnosis. This tool should facilitate sanitary checks and should also serve research in immunopathology.

Conclusions

The study of bonamiasis is an important example of the recent research developments in molluscan pathology and should continue to provide meaningful information. Our studies of bonamiasis, conducted both in the field (descriptive and

analytical epizootiology) and in the laboratory (experimental pathogenesis), have resulted in the following developments: (1) progress in conceptual approaches in the domain of marine molluscan pathology, (2) establishment of in vivo and in vitro experimental models, (3) proposals to check the spread of the disease and to permit continued breeding despite the presence of the parasite, (4) increased awareness within the edible oyster farming industry and government agencies of the need for measures to prevent the spread of disease, and (5) cooperation between international research teams concerned with epizootic diseases of molluscs. One work–study group has already met in France in April 1987.

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