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BONAMIA OSTREAE, PARASITE OF THE EUROPEAN FLAT OYSTER, *OSTREA EDULIS*, DOES NOT EXPERIMENTALLY INFECT THE JAPANESE OYSTER, *CRASSOSTREA GIGAS*

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Introduction

The protozoan *Bonamia ostreae (Ascetospora) is* the etiological agent of bonamiosis, or hemocyte disease of the European flat oyster, *Ostrea edulis* (Pichot *et al.,* 1980). Since this infection was first reported from the coast of Brittany (Comps *et al.,* 1980), the parasite has spread rapidly along the French Atlantic coasts (Grizel & Tigé, 1982), and in the European countries including the UK, Holland, Ireland and Spain (Bannister & Key, 1982; Van Banning, 1982; Polanco *et al.,* 1984). Under the EEC Council Directive 91/67, this parasitic protozoan is included in list II of Annex A. Serious pathogen agents for molluscs that should be obligatorily declared are placed in this list. Thus, flat oysters and other bivalves coming from an area infected by *Bonamia ostreae* are not permitted to be exported to a zone free of this parasite until there has been a demonstration of the absence of healthy carriers among these molluscs species. However, some bivalve mollusc species have been recognized by the European Commission as non carrier species for the parasites *Bonamia ostreae* and *Marteilia refringens* (93/169/CEE). It is the case for the Japanese oyster, *Crassostrea gigas.* For this purpose, to study the non carrier status of the Japanese oysters in bonamiosis transmission and in order to obtain complementary information about the absence of *Bonamia ostreae* detection on all the histological slides of *Crassostrea gigas* fixed tissues, observed during 15 years along the Atlantic French coast (Grizel, per. comm.), several experiments were performed. Experimental reproduction of the disease among Pacific oysters was tested by inoculation of purified parasites into the pericardial cavity. By an other way, some assays were also performed to try to transmit bonamiosis from injected Japanese oysters to healthy European oysters.

Material and Methods

Two hundred and fifty healthy flat oysters, *Ostrea edulis,* 2-year-old, were obtained from French Mediteranean Sea, a non contaminated habitat. 250 farmed Japanese oysters, 2-year-old and originating from Marennes Oleron (Charente Maritime) where both parasites *Bonamia ostreae* and *Marteilia refringens* are present, were used for these experiments.

Fifty animals of each species were used to test by histological examination the absence of parasitic infection before the beginning of experiments. - 50 animals of both species without any treatment were placed in the same tank (tank A) and served as negative controls. - 50 European flat oysters were inoculated with 150,000 purified *Bonamia ostreae,* per animal in pericardic cavity and then maintened in a tank (tank B) with 50 healthy Japanese oysters. - 100 Japanese oysters were inoculated the same day with 150 000 purified parasites, *Bonamia ostreae,* per animal in pericardial cavity, then 50 of these oysters were immediately mixed with 50 healthy flat oysters (tank C), the 50 others were mixed with 50 healthy European flat oysters 48 hrs after the inoculation of the Pacific oysters (tank D).

For the experiments, the animals were kept six months in 5001 tanks and fed daily by adding algae. Mortality and parasite detection among dead animals were performed daily. Moreover, the parasite prevalence was determinated at the end of experiments. Parasite detection was performed on heart or gills smears for dead animals and on his-

Table 1. Detection of the parasíte *Bonamia ostreae* among oysters reared in tanks at the laboratory.

	Tank A		Tank B		Tank C		Tank D	
	O. edulis	*C. gigas*	*O. edulis*	*C. gigas*	*O. edulis*	*C. gigas*	*O. edulis*	*C. gigas*
Dead oysters	0/5	0/8	6/21	0/3	0/5	0/12	0/6	0/13
Sacrificed oysters	0/36	0/38	7/15	0/47	0/42	0/29	0/3	0/32
Total of infected oysters	0/41*	0/46*	13/36*	0/50	0/47*	0/41*	0/42*	0/45*

N.B. Missing animals (*) are dead oysters too putrefied for examination

Table 2. Mortalities reported among reared oysters.

	Tank A		Tank B		Tank C		Tank D	
	O. edulis	*C. gigas*	*O. edulis*	*C. gigas*	*O. edulis*	*C. gigas*	*O. edulis*	*C. gigas*
Number of survivors	36	38	15	47	42	29	36	32
Cumulative mortality (%)	28	24	70	6	16	42	28	36

tological sections among survivors following the usual protocols.

Results and Discussion

The data are summarised in Table 1 for the prevalence of the parasite *Bonamia ostreae* in animals originating from the different tanks and in Table 2 for mortality. All animals examined at the beginning of experiments were free of parasites. For European flat oysters, this result was expected because of animals origin (Mediteranean Sea) and for Japanese oysters, this observation confirms results obtained by all previous epidemiological surveys (Grizel, pers. comm., Tigé, pers. comm.). Among the oysters of both species maintened in tank A, no parasite were detected (Table 1).

Despite the prevalence (46.6%) recorded in surviving inoculated flat oysters from tank B at the end of the experiment, the presence of the protozoan *Bonamia ostreae* or anything resembling this parasite was not found in the hemocytes or any of the examined organs of mixed Japanese oysters after a six month contact (Table 1). No parasite detection was possible among the Japanese oysters inoculated with 150,000 purified parasites *Bonamia ostreae* in tanks C and D. Moreover, no infection was detected among flat oysters mixed with inoculated Japanese oysters in these tanks after six months (Table 1), but *Bonamia ostreae* was observed in the inoculated flat oysters (Table 1). Out of 36 flat oysters exposed, 13 were found to be positive. This result was expected, because the injection of 150 000 purified parasites per oyster corresponds approximate to the LD_{50} value determinated by Hervio (1992).

High mortalities were observed in tank B among European flat oysters (Table 2), three months after inoculation. This result was comparable with data reported by Hervio (1992). Some important mortalities were also noted in tanks C and D (42% and 36%) among Japanese oysters, but no parasite infection was detected for these animals. Moreover, these mortalities appeared quickly after inoculation of parasites(1-5 days). Thus, this phenomen could be related to a too long magnesium chloride bath for anesthesia of these animals and to a high sensitivity of the species *Crassostrea gigas* to this substance. It seems impossible to induce an experimental infection by inoculation of purified parasites among Pacific oysters as it was described by Hervio (1992) and to transmit the protozoan to European

flat oysters by contact with inoculated Japanese oysters.

The present findings suggest that the parasite *Bonamia ostreae* infecting European flat oysters does not infect neighbour Japanese oysters. Under the same conditions, infections of healthy flat oysters can be obtained by proximity with infected flat oysters (Grizel, 1985). Moreover, the experimental reproduction of bonamiosis among Pacific oysters seems impossible by intracardial inoculation of purified parasites and thus, the transmission to healthy flat oysters is not observed after a six month contact with inoculated Pacific oysters. In conclusion, with these data, the Japanese oysters, *Crassostrea gigas,* should not be considered as a *Bonamia ostreae* susceptible species and as a possible carrier for this parasite. Indeed, these results confirm the previous observations obtained by the epidemiological surveys, the reports about *in vivo* experimental infections (Hervio, 1992) and the fine comparative *in vitro* study on the relationship between the parasite *Bonamia ostreae* and the hemocytes of *Ostrea edulis* and *Crassostrea gigas* (Chagot *et al.,* 1992).

Summary

The European flat oyster, *Ostrea edulis,* experimentally infected by the parasite *Bonamia ostreae* and healthy Japanese oysters, *Crassostrea gigas,* were cutlivated in the same tank for a six month period. After examination of dead animals heart or gills smears or survival oysters histological sections, no presence of *Bonamaia ostreae* was detected in the Japanese oysters. Moreover, Pacific oysters inoculated with purified parasites were held in tanks with healthy flat oysters. No parasite was found among animals of either species after six months.

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