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Myxidium leei (Myxozoa) infections in aquarium-reared Mediterranean fish species

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ABSTRACT: An episode of parasitic enteritis causing trickling mortalities at an exhibition aquarium reproducing Mediterranean ecosystems was found to be caused by the myxozoan parasite *Myxidium leei* Diamant, Lom & Dykova 1994. The myxozoan was recorded in 25 different fish species belonging to 16 Genera, 10 Families and 4 Orders. It was mainly detected in the intestine of affected fish, and was responsible for severe chronic enteritis. The parasite was probably introduced into the facilities with infected wild fish, and transmitted directly from fish to fish by cohabitation, transfer of infected material and necrophagia. Fish belonging to the Families Labridae and Blenniidae appeared as most susceptible, and the incidence of infections in members of the Sparidae was low. This study significantly widens the host spectrum for this virulent parasite and now includes many ubiquitous coastal Mediterranean species. Wild fish may have a significant role in the transmission of myxidiosis of cultured sparid fish.

KEY WORDS: *Myxidium leei* · Mediterranean fish · Aquaria · Infection

INTRODUCTION

Myxidiosis due to *Myxidium leei* is one of the most severe expanding parasitic diseases of cultured Mediterranean fish species (Le Breton 1999). Serious losses in cultured sparids such as gilthead sea bream *Sparus aurata*, sharpsnout sea bream *Puntazzo puntazzo* and red sea bream *Pagrus major* have been attributed to infections by this myxozoan since 1991–1992 (Diamant 1992, LeBreton & Marques 1995, Sakiti et al. 1996). *M. leei* infections have also been recorded from wild mullets (*Liza aurata*, *L. ramada*, *Mugil saliens* and *Chelon labrosus*) captured close to sea bream growing farms (Paperna 1998) and from cultured red drum *Sciaenops ocellatus* after cohabitation with infected gilthead sea bream (Diamant 1998).

Considering this low degree of host specificity and the existence of direct fish to fish transmission of myxidiosis (Diamant 1997, 1998), the dynamics of the infections in open or multi-specific systems can become extremely complex. Nevertheless, the possible role of infected wild fish in the epizootics of this disease or in its transmission to cultured fish still remains to be evaluated. *Myxidium leei* has hitherto been described in fish held in the Mediterranean area including the northern Red Sea, but 2 other similar enteric Myxosporea have been reported in anemone fish *Amphiprion frenatus* held in the Pacific Coast of the USA (Kent 1999) and in turbot *Scophthalmus maximus* grown along the Atlantic Coast of NW Spain (Branson et al. 1999).

In the present work, infections by *Myxidium leei* in 25 different fish species (belonging to 4 Orders and 10 Families) reared in an exhibition aquarium are studied. Histopathological and parasitological observations are reported and the possible implications of our findings on the host/parasite relationships and on the disease epizootiology are discussed.

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MATERIALS AND METHODS

From January 1999 to June 2000, trickling mortalities affecting different fish species were noticed at several exhibition tanks of 'L'Aquàrium', Barcelona, Spain. This large recreational aquarium maintains 2 systems, 'Mediterranean' and 'Tropical', which receive completely independent water supplies. The Mediterranean system facilities include several 5 to 90 m^3 tanks reproducing different Mediterranean coastal ecosystems, quarantine and stock tanks, and a large 3700 m^3 'Oceanarium'. Fish from this system are held in seawater (39‰ salinity, 18 to 19°C), with common filtration equipment, and fed a mixture of commercial food, whole sardine, mackerel, blue whiting, mussel, shrimp, squid and spinach. Effluent water from exhibition and quarantine facilities is actively disinfected with ozone treatment (redox: 700 mV).

All the specimens studied were captured from the wild (NE Spanish Mediterranean Coast). After a variable quarantine period (2 wk minimum) the fish were introduced into the exhibition tanks. Once in the exhibition facilities, fish were occasionally moved between tanks according to the program requirements. In most cases this fact and the lack of individual identification did not allow us to know the time of residence of each affected fish in the facilities.

Affected fish were removed from the aquaria and necropsies were carried out immediately. Scrapings of the intestinal mucosa were obtained and observed under the light microscope. When the presence of myxozoan spores was confirmed, samples of different levels of the digestive tract were fixed in 10% phosphate buffered formalin for histopathological studies and preserved in 70% ethanol for parasitological examination. Samples for histopathological studies were serially dehydrated and embedded in paraffin using standard procedures. Histological sections (4 to 5 µm) were stained with hematoxylin-eosin or Gram stains. Samples preserved in alcohol were rehydrated in Hanks' Balanced Salt Solution (HBSS) and mounted on glass slides for examination and measuring of spore dimensions by light microscopy.

RESULTS

A total of 349 carcasses or moribund fish were examined during the period studied. In 66 fish belonging to 25 different species (Table 1), an intestinal myxozoan was diagnosed in fresh smears of the digestive tract which contained large numbers of sporoblasts and mature spores (Fig. 1). The morphology of these spores resembled that of the myxozoan *Myxidium leei* Diamant, Lom & Dykova, 1994. Measurements of spores obtained from 7 different host species are given in Table 2. More than 67% of the total necropsies carried out at the Mediterranean facilities corresponded to species where *M. leei was* detected. *M. leei* infection was confirmed in fish belonging to 16 Genera, 10 Families and 4 Orders (Table 1). All of the species listed except *Sparus aurata* represent new host records for this parasite. The infection was restricted to different tanks and facilities belonging to the Mediterranean system and was never detected in any of the fish from the tropical system examined during the same period (n = 460). Some of the infected fish had been maintained in the exhibition tanks for longer than 1 yr when they died (up to 3 yr in 1 case), although the culture conditions and the moves between tanks did not allow further tracking of the individual route of infection. As determined by the percentage of necropsies positive for the myxozoan, the frequency of infection among Labridae fish and the high mortality caused among Blenniidae was noticeable. It must also be noted that several cases were detected among non-symptomatic animals, which were sampled as controls or died accidentally. These included the only 2 cases registered in *Diplodus* spp.

In comparison, sparids were rarely found to be infected (Table 1) although more than 200 other individuals belonging to the Sparidae were present in the same tanks. Furthermore, 52 necropsies carried out on different specimens of *Oblada melanura*, *Pagellus acarne*, *P. bogavareo*, *P. erythrinus*, *Spondyliosoma cantharus*, *Diplodus puntazzo*, *D. annularis*, *Sarpa salpa*, *Boops boops* and *Lithognathus mormyrus* were negative for the presence of *Myxidium leei*.

Pathological observations of the affected fish included severe cachexia and distended abdomen in some individuals. Frequently, the carcasses showed evidence of necrophagia by other tank mates. In the

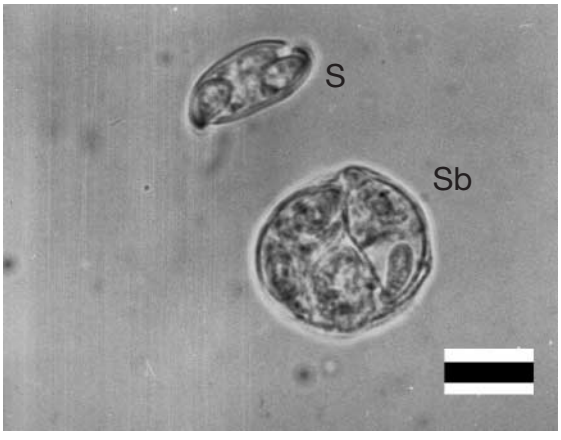

Fig. 1. Sporoblast (Sb) and spore (S) of *Myxidium leei* from a wet mount of a gut scraping from infected *Symphodus mediterraneus*. The gut sample was preserved in ethanol and re-hydrated in HBSS. Scale bar = 10 µm

Table 1. Epizootiogical data for the cases of myxidiosis diagnosed in the aquarium. Only data from tanks and from fish species in which the infection was confirmed during the experimental period are included. Data on the population of each species are only approximate and inferred from periodical visual counts at the exhibition tanks. Data from the 'Oceanarium' are fragmentary because records of mortalities and populations are not available and recovery of carcasses is only occasional

Fish species	Exhibition and quarantine tanks				Oceanarium	
	Estimated population	Estimated total fish losses (%)	No. of necropsies	Necropsies +*Myxidium leei* (%)	No. of Necropsies	Necropsies +*Myxidium leei* (%)
Labridae						
Coris julis	579	219 (37.8)	32	3 (9.4)		
Symphodus tinca	90	27 (30)	23	9 (39.1)		
S. ocellatus	16	1 (6.25)	1	1 (100)		
S. mediterraneus	23	17 (73.9)	15	6 (40)		
S. rostratus	53	20 (37.7)	13	2 (15.4)		
S. roissali	19	7 (36.8)	5	1 (20)		
S. cinereus	5	2 (40)	1	1 (100)		
S. melops	1	1 (100)	1	1 (100)		
Thalassoma pavo	64	20 (31.2)	8	3 (37.5)		
Labrus viridis	21	5 (23.8)	5	1 (16.7)		
Labrus merula	9	7 (77.8)	7	7 (100)		
Labrus bergylta					1	1 (100)
Xyrichtys novacula	3	2 (66.6)	2	1 (50)		
Subtotal Labridae	883	328 (37.1)	113	36 (31.58)	1	1 (100)
Centracanthidae						
Spicara maena	61	37 (60.6)	29	1 (3.4)		
Sparidae						
Sparus aurata					8	1 (12.5)
Diplodus sargus	3	3 (100)	3	1 (33.3)		
Diplodus vulgaris	40	1 (2.5)	1	1 (100)		
Subtotal Sparidae	43	4 (9.3)	4	2 (50)		
Molidae						
Mola mola					1	1 (100)
Mullidae						
Mullus surmuletus	268	94 (35.1)	42	2 (4.8)		
Batrachoididae						
Halobatrachus didactylus	6	2 (33.3)	2	2 (100)	2	2 (100)
Pomacentridae						
Chromis chromis	66	3 (4.54)	3	1 (33.3)		
Blenniidae						
Lipophrys pavo	1	1 (100)	1	1 (100)		
'Blennids' [a]	52	52 (100)	16	13 (81.2)		
Subtotal Blenniidae	53	53 (100)	17	14 (83.3)		
Gobiidae						
Gobius niger	58	14 (24.1)	6	2 (33.3)		
Scorpaenidae						
Scorpaena porcus	142	17 (12)	7	1 (14.3)		
Totals	1580	552 (34.9)	223	61 (27.2)	12	5 (41.7)
Totals exhibition (Mediterranean), Quarantine tanks + Oceanarium	Total number of necropsies (*M. leei* and no-*M. leei*-affected species): 349 Number of necropsies (*M. leei*-affected species): 235 (223 + 12) Necropsies positive (+) to *M. leei*: 66 (61 + 5)					

[a]Fish not classified to the species level during the necropsies and considered as a collective 'blennids' group in the total count. The group actually consisted of different specimens of the genera *Blennius*, *Parablennius* and *Scartella*. All the animals in this tank died and their necropsies were positive for *M. leei*

Table 2. Comparison of morphometrical data from different reports of *Myxidium leei* and similar myxozoans. Average values are given with standard deviation when available. Range values are given in parentheses

Myxozoan Host	Spore dimensions Length	Spore dimensions Width	Polar capsules Length	Polar capsules Width	Polar capsules Coils	Source
Myxidium leei						
Sparus aurata	14.7 (13.2–15.2)	6.9 (5.6–7.8)	7.4 (6.2–8.8)	3.2 (2.8–3.8)	7 (6–8)	Diamant et al. (1994)[a]
Sparus aurata	16.2 (15.2–17.7)	7.8 (7.5–8.6)	8.2 (6.9–9.8)	3.6 (3.1–4.3)	?	Diamant (1998)
Sparus aurata	16.7 ± 1 (15–18)	9.7 ± 1 (8–11)	8.6 ± 1.3 (8–9)	2.8 ± 0.8 (2.5–4)	6–8	Saikiti et al (1996)
Sparus aurata	15–18	7–8	7	3	?	Diamant (1992)[a]
Diplodus puntazzo & Pagrus major	15–19	5–7	6.5–9	2.5–4	?	LeBreton & Marques (1995)
Sciaenops ocellatus	17.5 (15.5–9.5)	7.4 (7.0–8.7)	8.4 (7.0–9.8)	3.8 (3.3–4.5)	?	Diamant (1998)
Symphodus tinca	17.5 ± 1.6 (14.4–19.6)	8.6 ± 0.5 (8.1–9.2)	8.3 ± 0.7 (8.1–9.2)	3.3 ± 0.3 (2.9–4.03)	?	Current study[b]
S. mediterraneus	17.5 ± 1.1 (16.1–19)	8.2 ± 0.6 (7.5–9.2)	7.8 ± 0.6 (6.9–8.6)	3.2 ± 0.4 (2.5–3.5)	7–8	Current study[b]
Sparus aurata	17.7 ± 0.6 (16.7–18.4)	8.2 ± 0.4 (7.5–8.1)	7.7 ± 0.3 (7.5– 8.1)	2.9 ± 0.4 (2.3–3.5)	?	Current study[b]
Halobatrachus didactylus	16.8 ± 1 (16.1–18.4)	8.2 ±1 (6.9–9.2)	7.2 ± 0.3 (6.9–7.5)	3.1 ± 0.3 (2.9–3.5)	?	Current study[b]
Scartella cristata	15.9 ± 0.9 15.0–17.8	8.5 ± 0.6 (7.5–9.2)	7.8 ± 0.5 (6.9–8.1)	3.2 ± 0.3 (2.9–3.5)	?	Current study[b]
Coris julis	17.1 ± 0.7 (16.1–18.4)	8.4 ± 0.7 (7.5–9.2)	7.4 ± 0.5 (6.9–8.1)	2.7 ± 0.3 (2.3–3.1)	?	Current study[b]
Diplodus sargus	16.16 ± 1.1 (14.4–17.3)	6.8 ± 0.2 (6.3–6.9)	7.4 ± 0.5 (6.9–8.1)	3.0 ± 0.4 (2.3–3.5)	?	Current study[b]
Unidentified *Amphiprion frenatus*	11	6.6	4.5	2.5	?	Kent (1999)[c]
Unidentified *Scophthalmus maximus*	(20–25)	(11–14)	(9–13)	(3.6–6)	(10–13)	Branson et al. (1999)

[a]Material fixed in formalin
[b]Material fixed in ethanol and re-hydrated in HBSS
[c]Paraffin-embedded histological sections

necropsies, thickening of the intestinal wall and occasional haemorrhages were commonly observed. Histopathological observations generally showed severe chronic enteritis due to the presence of a large number of different developmental stages of the parasite (Fig. 2). The intestinal epithelium was clearly altered and inflammation, congestion and haemorrhages in the submucosa were occasionally noticed. Myxozoan spores and cellular debris were also observed within the intestinal lumen.

DISCUSSION

A myxozoan parasite was detected in the intestine of dead or moribund fish held in a large recreational

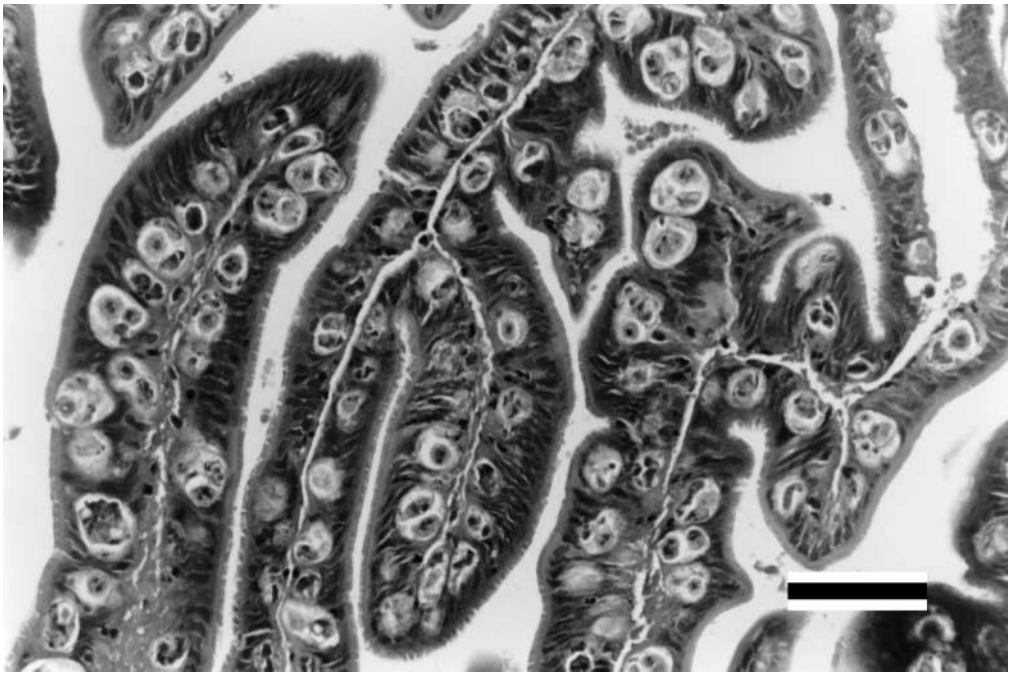

Fig. 2. Histological section of a *Myxidium leei*-infected gut of *Scartella cristata*. Scale bar = 50 µm

aquarium, in tanks reproducing different Mediterranean coastal ecosystems. Morphometry of the mature spores isolated from 7 different fish species coincides with that of *Myxidium leei* Diamant, Lom & Dykova, 1994. Minor metric differences were detected between the spores obtained from different hosts in our study and between these and some of the reports of *M. leei* by other authors (see Table 2). Nevertheless, the rank of sizes recorded for *M. leei* in the literature is rather wide and all our material fits well in the species variability. Methodological differences might account for this variability. This notwithstanding, Diamant (1998) studied the transmission of *M. leei* between *Sparus aurata* and *Sciaenops ocellatus* and reported differences in the size of the spores isolated from each of the 2 fish species, which would suggest some influence of the host species in the final size of the parasite. Two other myxozoans resembling *M. leei* have been recently reported, one from cultured turbot (Branson et al. 1999), the other from a tropical anemone fish held in North America (Kent 1999). The species reported from turbot is clearly larger and its polar filament has more coils (Table 2). The latter appears smaller and, moreover, might well be a different species, considering that it infected a tropical fish and that the infections in our study have never been detected in the 'Tropical' facilities. However, *M. leei* has become established in the tropical marine region of the northern Red Sea due to an accidental introduction (Diamant 1997), indicating that a potential transmission to tropical fish species should not be underestimated. Another enteric myxozoan resembling *M. leei* has also been recently found in tiger puffer *Takifugu rubripes*, from Japanese fisheries (Tun et al. 2000).

Our histopathological observations of the digestive tract of the fish affected by the parasite were similar to previous descriptions of enteric lesions due to myxozoans (Diamant 1992, Diamant et al. 1994, Branson et al. 1999). The severity of the lesions found in the present study points to myxidiosis as the cause of morbidity in the aquarium-reared fish.

Myxidium leei infection has been described so far in cultured sparids (Diamant 1992, Diamant et al. 1994, Le Breton & Marques 1995) and sciaenids (Diamant 1998) as well as in wild mullets sampled at the vicinity of gilthead sea bream growing farms (Paperna 1998). Our results substantially increase the spectrum of species susceptible to be infected by the parasite and demonstrate that *M. leei* has a very low degree of host specificity, a fact that seems rather unusual among the Myxozoa. From our data it is not possible to elucidate whether the affected fish were infected in the wild or they acquired the infection in the aquarium. However, the overall data support one or more introductions of infected fish from the wild and then secondary spreading of the infection due to movements of sub-clinically infected individuals, water, or both. Chronologically the infection was detected first in labrids (*Labrus* spp. and *Symphodus* spp.), which might point to some of these as responsible for the primary introduction of the infection into the facilities. Nevertheless, one can argue that this could only reflect the higher suscepti-

bility of these species to the myxidiosis and that a more resistant non-symptomatic carrier might be involved in the transmission of the disease.

Although the cases of myxidiosis acquired within the aquarium facilities point to a direct fish to fish transmission of the parasite, the existence of more complex cycles cannot be disregarded. Myxosporean/actinosporean alternating life cycles are known for an increasing number of freshwater species (Lom & Dyková 1995). Such cycles have yet to be described in marine environments although actinosporeans have already been found from marine oligochaetes (Roubal et al. 1997), polychaetes (Køie 2000) and sipunculids (Ikeda, 1912). Given the existence of complex communities reproducing Mediterranean ecosystems in the aquariums, heteroxenous cycles would be theoretically possible. However, coprophagy, necrophagia and predation are common in the exhibition tanks, especially in the 3700 m^3 '*Oceanarium*', where sick fish are only occasionally recovered for necropsies (Table 1) due to high predatory activity. This would favour direct fish to fish transmission through ingestion of developmental stages of the parasite, a route which is known to be effective in the transmission of *Myxidium leei* (Diamant 1997).

The observed low incidence of *Myxidium leei* infections detected in gilthead sea bream and other sparids at the aquarium, although they were abundant in some of the affected tanks is remarkable. However, 2 non-symptomatic *Diplodus* spp. were found to be infected after they died accidentally. These observations could suggest a lower susceptibility of these fish, if compared to some labrids or blennids. Differential susceptibility to this myxidiosis has been reported among sparids cultured in netpens at the Eastern Mediterranean (Athanassopoulou et al. 1999, Rigos et al. 1999), with *Diplodus puntazzo* (*Puntazzo puntazzo*) being the species suffering the highest mortality rates. Although water temperature or fat contents in the diet have been suggested to explain such differences (Rigos et al. 1999), other modulators of the host-parasite relationships such as immune mechanisms, physiological status and ethological and ecological factors should be considered as well.

Further investigations are needed to confirm the occurrence of this parasite in wild stocks. This notwithstanding, several species affected during this episode are ubiquitous in the Mediterranean coasts and therefore might play a relevant role in the transmission of *Myxidium leei*. The existence of natural reservoirs in the wild would have obvious trascendence in the case of netpen culture of Sparidae fish, in ecosystems in which those reservoirs are abundant. Thus, application of strict preventive measures and efficient early diagnosis are recommended to prevent dissemination of the disease.

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