地中海西部の畜養クロマグロにおける住血吸虫寄生

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Blood Fluke Infection of Cage Reared Atlantic Bluefin Tuna *Thunnus thynnus* in West Mediterranean

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ABSTRACT—Infection of a blood fluke, possibly *Cardicola* sp. (Digenea: Aporocotylidae), in reared Atlantic bluefin tuna *Thunnus thynnus* was investigated. Parasitological analyses included visual examination of the heart for the presence of adult fluke and stereomicroscopic and histopathological analyses of the gill to assess the presence of lesions caused by parasite eggs. No adult flukes were found in the hearts. Some of the gills exhibited small white to yellow foci involving single filaments. Blood fluke eggs were found in gill tissue sections of 29.6% of sampled tuna. A slight inflammatory response was observed around most of these eggs, while occasionally individual eggs were encapsulated by a granulomatous reaction. Despite the absence of remarkable pathological effects in the infected tuna, blood flukes combined with other agents may cause major problem.

Key words: *Thunnus thynnus*, Atlantic bluefin tuna, *Cardicola*, blood fluke, Aporocotylidae

The Atlantic bluefin tuna (BFT) (*Thunnus thynnus*) is a fish exploited by commercial fishing during centuries in the entire Mediterranean area. Due to the increasing demand for this fish by the sashimi-sushi market, a "capture-based" aquaculture industry has been developed in the Mediterranean Sea over the last decade. This activity involves the capture of adult individuals, during the months of May-June, which enter the Mediterranean Sea for reproduction. After capture, fish are introduced in floating cages where they stay for 2 to 10 months (average of 6 months) fed with highly fat content fish, mainly chub mackerel *Scomber japonicus*, Atlantic mackerel *Scomber scombrus*, European pilchard *Clupea pilchartus* and round sardinella *Sardinella aurita*. After this period, tunas are sacrificed in the floating cages and immediately commercialized fresh or frozen.

Among pathological problems reported in reared tuna, a blood fluke *Cardicola forsteri* (Digenea: Aporocotylidae), has been pointed out as a significant risk of tuna health[1]. Initially identified in the Australian population of farmed southern BFT *Thunnus maccoyii*[2], this blood fluke was later reported in Atlantic BFT[3–5], being the only one aporocotylid reported so far in this species. Aporocotylids are parasites of marine and freshwater fish[6]. Most species are located in the heart, bulbus arteriosus, ventral aorta or branchial vessels, although the cephalic or dorsal vessels are not uncommon habitats[7]. Once established, adult flukes lay eggs which travel to the gills where they lodge. The eggs hatch there and break out of the gill as free living miracidia[6]. These miracidia infect an intermediate host into which they penetrate to undergo asexual reproduction. Bivalves and polychaetes have been reported to be intermediate hosts for some marine aporocotylids[6,8]. Cercariae emerge from the intermediate host and actively search for the definitive host, a fish, penetrate the skin of the host and juvenile flukes attempt to reach the circulatory system in which they undergo a migration to the final site where they mature[6].

Blood flukes are suspected cause of mortalities in a number of farmed fish species, for example amberjack *Seriola dumerili* in Spain[9] and tiger puffer *Takifugu rubripes* in Japan[10]. These mortalities are generally considered to be due to the eggs of the blood flukes, which block capillaries in gill lamellae and other organs. Although Munday and Hallegraeff[11] briefly mentioned 'a moderately severe, multifocal, granulomatous myocarditis' associated with the presence of eggs (presumably of *C. forsteri*) during a mortality of southern BFT in 1996, more intensive histopathological investigations of the heart, gills and other organs of ranched southern BFT have not yet established a clear and consistent link between the burden of eggs of *C. forsteri* and mortality (Valdenegro and Nowak, unpublished data). Recently, Hayward *et al.*[12] associated high number of blood flukes with increased lysozyme and decreased haemoglobin levels in southern BFT. According to these authors blood flukes might also be associated with the onset of elevated mortalities.

Cardicola forsteri infection is present in wild Atlantic[13] and southern[14] BFT at low levels, and it is exacerbated during the rearing cycle in confined conditions[10,15]. In fact, Aiken *et al.*[16] suggest that the infection in southern BFT occurs in the farming zone. *C. forsteri* intensities and prevalences are very low in

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the wild[14] and at transfer[17] while an antibody response against *C. forsteri* is only initiated after transfer into the farming zone from the wild[14]. As the impact of aporocotylids in cage reared tuna must not be neglected, the objective of the present work was to study the prevalence of blood fluke infection in BFT reared in west Mediterranean, in the Murcian coasts.

Materials and Methods

Between November 2006 and December 2008, a total of 108 Atlantic BFT from six BFT farms located in Murcia region (South East Spain) were sampled. Two farms are located in San Pedro del Pinatar (37°49′ 55.46″ N, 0°39′42.33″ W) while the remaining farms are located in "El Gorguel" (Cartagena, 37°34′31, 692″N, 00°52′30, 702″W). Tuna weights in the range of 27–40 kg, 150–210 kg and 240–300 kg for 77.4%, 18.3% and 4.3% of the animals, respectively (Table 1). Fish were shot underwater and then hoisted by hook. Gills and hearts were removed on board immediately after tuna were slaughtered. The first gill arch was obtained from each tuna. For histology, two to four 1 cm long pieces of the gill arch were cut out and immediately fixed in 10% neutral buffered formalin. The rest of the gill arch and the heart of each animal were collected in individual plastic bags, transported to the laboratory and stored at –20°C, until examined with a stereomicroscope (Nikon, SMZ 800) for parasite eggs and pathological changes. To do this, approximately, 120–150 gill filaments per arch were individually observed under the stereomicroscope, placed in a large Petri dish with saline solution. Hearts were dissected and flushed with water to dislodge any adult flukes and flushes were then poured into Petri dishes and similarly examined. To carry out the histopathological study, the fixed pieces of gill arch (2–4 pieces per tuna) were decalcified in a mixture of 5% EDTA and 3% HCl for 24 h, followed by 60 min washing in fresh water, prior to performing routine histological processing as follows; samples were embedded in paraffin blocks, cut and stained with haematoxylin and eosin (H & E). The sections were examined under a microscope (NIKON Eclipse 50i) and lesions were photographed.

Epilnfo 2002 (CDC) and SAS (SAS Institute) were used to do statistical analysis. Yate's corrected chi-squared test was used to compare the frequency of blood fluke egg positive tuna across independent categorical variables (Table 1) and the non-parametric Kruskal-Wallis tests was employed to compare mean blood fluke egg densities. Logistic regression was used to investigate the relationship between tuna infection (Table 2) and body weight, adjusted for farm. Other variables including farm location, month and time in captivity were not included in the model because of their strong correlation with each other and with Farm, leading to model convergence failure[18]. The maximum likelihood estimation model was used, Ps were from likelihood-ratio chi-squared test and alpha was 5% ($p < 0.05$) level for a double-sided test.

Table 1. Percentage of captive bluefin tuna individuals positive for blood fluke eggs in Spain in 2006–07

Variables	No. of fish	% positive
Location		
Cartagena	71	31
San Pedro	37	27
Farm		
San Pedro		
1	20	5*
2	17	53
Cartagena		
1	30	43
2	6	50
3	15	33
4	20	5
Time in captivity (months)		
3	6	50
4	37	27
5	50	28
6	15	33
Sampling month		
October	6	50
November	37	27
December	50	28
January	15	33
Body weight (kg)		
27–40	81	20*
150–210	16	69
240–300	5	40

* Significant differences between variable levels at $p < 0.05$.

Table 2. Estimates from the logistic regression models of blood fluke infection in captive bluefin tuna in Spain

Variables	Odds ratio	95% Confidence intervals	*P value*
Body weight (Kg)			
27–40	1.00		
150–300	2.61	6.91, 0.99	0.0543
Farm			
Cartagena			
1	1.00		
3	2.23	8.18, 0.61	0.2270
4	0.59	5.34, 0.06	0.6369
San Pedro			
1	0.16	1.42, 0.02	0.1016
2	2.37	6.42, 0.87	0.0919

Results

Hearts were macroscopically normal and no adult parasites were observed. Some of the gills exhibited small white to yellow foci involving single filaments possibly associated to *Cardicola* sp. infection. Histopathology revealed the presence of eggs consistent with those described for aporocotylids[6], in gills of 29.6% of tuna sampled (Table 1). The number of eggs in infected filaments was low (≤ 10 eggs), medium (11–21 eggs) and high (≥ 22 eggs) in 46.3%, 43.9% and 9.8% of infected gill samples, respectively. The Kruskall Wallis test indicated that egg density was highest in gill samples with the greatest number of infected filaments ($p < 0.05$). The percentage of tuna positive for eggs were significantly greater in 150–300 kg compared to 27–40 kg tuna and differed between farms ($p < 0.05$) (Table 1). There were no statistically significant differences in the percentage of infected tuna according to time in captivity or sampling month ($p > 0.05$). In the logistic regression analysis, infection was marginally significantly greater in 27–40 kg tuna compared to heavier tuna (odds ratio = 2.61, p = 0.0543) (Table 2) and there was similar differences between farms (Table 2).

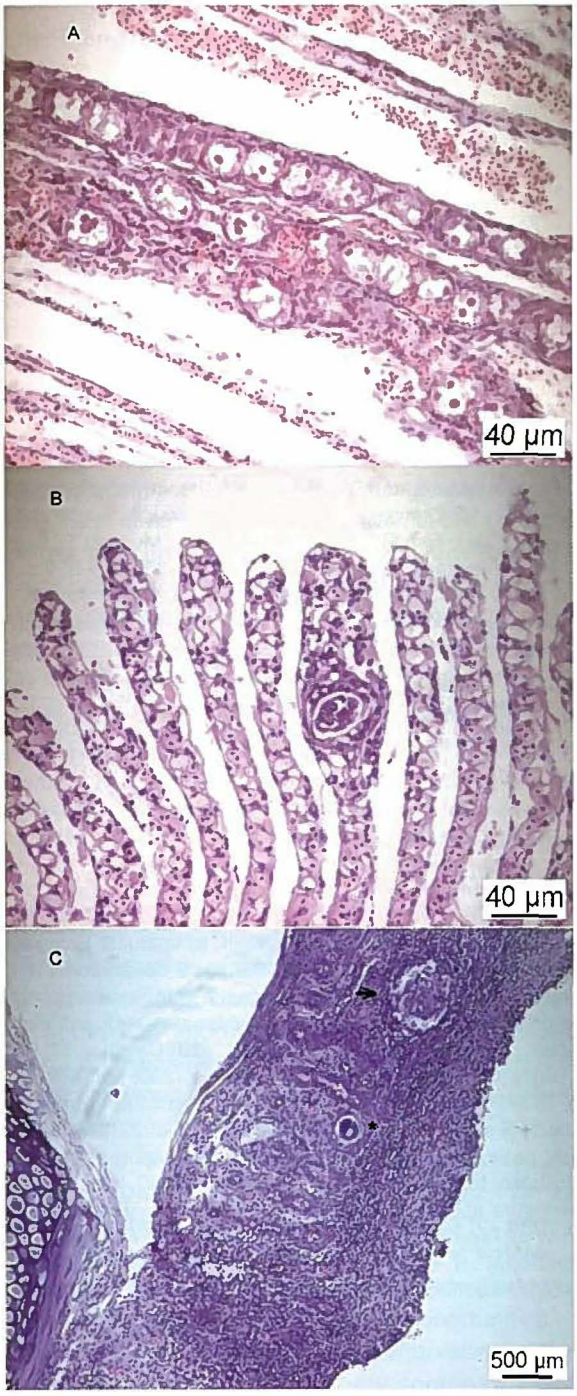

Fig. 1. Blood fluke eggs in the gills of Atlantic bluefin tuna (H&E). A. Different degrees of miracidial development. B. Secondary gill lamellae with an individual egg. C. Typical inflammatory response to an egg (asterisk), and a focal granuloma (arrow) formed around an individual egg.

Eggs found in the gill displayed different degrees of miracidial development (Fig. 1A). A slight inflammatory response was observed around most of the eggs (Fig. 1A, B) while very occasionally, individual eggs were encapsulated by a granulomatous reaction (Fig. 1C), which consisted of epithelioid cells and lymphocytes surrounded by fibroblasts and fibrocytes in older lesions. Some of the eggs were observed in afferent filamentary arteries but most of them had traversed the capillaries of the gill lamellae and therefore observed within the lamellae.

Discussion

Infection of a blood fluke, possibly *Cardicola* spp., in reared Atlantic bluefin tuna was investigated. Although *C. forsteri* is the only blood fluke identified from Atlantic BFT, *Cardicola* sp. with a different ITS2 sequence has been reported from Atlantic BFT in Spain[14,19] which strongly suggests co-existence of at least two species of blood flukes in Atlantic BFT. The prevalence of eggs detected in the present study was similar to the prevalence reported by Deveney *et al.*[20] in southern BFT who observed a 31% of infection. Mladineo[4] detected *C. forsteri* eggs, but no adult flukes, in 63.34% of sampled Atlantic BFT harvested in the Adriatic Sea. Differences in the prevalence between these studies could be due to the fact that some of the tuna sampled in the latter study were specimens that were found dead in an Adriatic tuna farm associated to other pathogens which may have predisposed to *C. forsteri* infection.

Even though tuna sampled in the present study showed a high number of eggs and disseminated granulomas without isolation of any adult parasite, this finding remains only an assumption of possible *Cardicola* spp. infection. Other authors have also failed to find adult forms of *C. forsteri* in Atlantic BFT even though eggs

were found in gills[4,6,17]. They observed that as water temperature decreased the intensity and prevalence of *C. forsteri* infection of southern bluefin tuna declined after an initial peak to an average of 3.1 flukes per host and a prevalence of 35% respectively. Similar declines in intensity and/or prevalence following a peak has been observed in other cultured fish species infected by aporocotylids. Ogawa *et al.*[21] investigated infection of amberjack *Seriola dumerili* blood flukes *Paradeontacylix* spp. and suggested that the blood fluke infection has an annual cycle; cercarial invasion starts in September, eggs accumulate in the gills and heart from November, and mortality, occurring in the winter months from December to March, decreases with increasing water temperature. Except six fish sampled in October, all tuna used in the present study were sampled in winter months. The lack of adult blood fluke could be due firstly, to the low temperatures and secondly, to their hypothetical establishment in other locations not sampled in this study. Indeed, Aiken *et al.*[16] reported the presence of *C. forsteri* in branchial arteries of southern BFT while *Sanguinicola inermis* adults were observed in the afferent branchial arteries, ventral aorta and bulbous arterious[7] of common carp *Cyprinus carpio*.

As mentioned above, Aiken *et al.* suggested that fish commonly become infected with *C. forsteri* once in captivity[16]. In the present study, infection was not related to duration in captivity but to the size of the host, greater in 150–300 kg tuna than in 27–40 kg. The apparent negative correlation between infection and weight or age requires further investigation. Infection accumulating with age would suggest some degree of parasite tolerance. On the other hand, although no correlation between fluke intensity and growth measured as condition index has been reported[17] a negative effect should be considered as white lesions that commonly occurred in the gills are presumed to be caused by the lodgement and hatching of blood fluke eggs[22]. Despite the pathology observed was not sufficient to lead to mortalities, it is possible that combination with other factors will be able to cause severe illness or death[15]. It is generally believed that a disease assists in secondary infections. Primary infection might compromise host biodefence mechanisms. Kumon *et al.*[23], for example, found that blood fluke-infested yellowtail *Seriola quinqueradiata*, when challenged with the bacterial fish pathogen *Lactococcus garvieae*, had a significantly higher final cumulative mortality than the fish uninfested with blood fluke. As blood fluke infection may combine with other agents to cause such effects further investigations into the epidemiology of this parasite are warranted.

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地中海西部で畜養された大西洋クロマグロにおける住血吸虫（おそらく *Cardicola* sp.）の寄生を実体顕微鏡観察と病理組織学的観察により調査した。29.6%のクロマグロの鰓に虫卵が見られたが，心臓には虫体は存在しなかった。鰓弁に白色から黄色の小病巣が見られる場合もあった。虫卵の周囲には軽度の炎症反応が見られ，肉芽腫性の反応が見られる場合もあったが，寄生されたクロマグロへの病理学的影響は軽微であった。

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