

First report of Marteiliosis in *Mytilus galloprovincialis* in Albania

*Primo caso di Marteiliosi in *Mytilus galloprovincialis* in Albania*

Aleksi Pëllumb^{1*}, Ceschia Giuseppe², Sulaj Kapllan¹

¹ Institute of Veterinary Research “Bilal Golemi” - Tirana, Albania

² Istituto Zooprofilattico Sperimentale delle Venezie, Laboratorio Patologia Molluschi - 33030 Basaldella di Campoformido (UD), Italy

SUMMARY - Marteiliosis, a mollusc disease caused by the protistan parasite *Marteilia* spp., has never been notified in Albanian mussel farming before this study, which is an integral part of bivalve molluscs sanitary monitoring for the year 2005.

Mytilus galloprovincialis is sensible to this disease and was the investigated species in this study. Marteiliosis is one of the shellfish diseases listed by OIE. Monitoring of this infection in mussel farming in Albania has been one of the recommendations of EU, focusing on export of shellfish, for human consumption or for aquaculture, in EU market.

Samples of *Mytilus galloprovincialis* were collected from Butrinti Lagoon (South of Albania) and analysed by cytological (300 mussels) and histological (150 mussels) methods. 19.3% of animals tested with cytological method and 10% of animals tested with histological method resulted infected by *Marteilia* spp.

In conclusion, this study highlighted the presence of this parasite in *Mytilus galloprovincialis*.

RIASSUNTO – La Marteiliosi, una malattia dei molluschi dovuta a *Marteilia* spp., non era mai stata segnalata nelle mitilocolture albanesi prima di questa ricerca, parte integrante del monitoraggio sanitario “Salute Animale” dei molluschi bivalvi per il 2005.

I mitili (*Mytilus galloprovincialis*), sono ritenuti specie sensibili a questo agente eziologico e pertanto inclusi nelle varie liste dell’OIE e della EU. Pertanto il monitoraggio programmato negli allevamenti è necessario ai fini di ottemperare alle richieste dell’EU per l’esportazione nei Paesi comunitari.

La ricerca è stata condotta sulla mitilocoltura presente nella Laguna di Butrinti (Albania meridionale). Da tre zone d’allevamento sono stati raccolti soggetti per essere sottoposti ad indagine di laboratorio mediante metodo citologico (300 soggetti) ed istologico (150 soggetti).

L’esito delle analisi è risultato positivo per *Marteilia* spp. per il 19,3% dei soggetti sottoposti ad esame citologico, mentre l’esame istologico è risultato positivo per il 10%.

Key words: *Marteilia* spp., *Mytilus galloprovincialis*, Albanian mussel farming, Butrinti lagoon.

* Corresponding Author: c/o Institute of Veterinary Research “Bilal Golemi”, Street Aleksander Moisiu 82 - Tirana, Albania. Tel/fax: 00355-4372912; E-mail: paleksi03@yahoo.co.uk.

INTRODUCTION

Albanian aquaculture consists on breeding of some ichthyic species of freshwater (carp and trout) and sea water (gilthead sea bream and European sea bass) and mussels, mainly represented by *Mytilus galloprovincialis*. Even though the artificial breeding, the fishing activity is developed in sea and lagoons. Focusing on molluscs, a very important role is given to their harvesting in *Donax* spp. natural production areas.

In Albania in 1991 were collected, from natural areas, 662 tons of *M. galloprovincialis* and 450 tons of *Donax* spp. (Kristo, 2003). In the following years, this production was reduced and only in the last years is going to increase. In 2005 mussels production, mainly originated from Butrinti lagoon, was evaluated over 1000 tons. In this lagoon there are 58 breeding modules, which occupy 1250 m² of the surface and are located in the northern, western and southern areas. This production system is known as overhanging cemented panels, with ropes of 2.5 m. length each, which are 40 cm distant between them. Molluscs species mentioned above are required by EU market. To export these species, Albanian government is going to accomplish the sanitary and hygienic conditions, provided for decisions 2003/804 and 2004/623 of EU Commission. For this purpose, Albania engaged competent authority to monitoring mollusc production areas for the presence of diseases and to evaluate the epidemiological situation for diseases included in the OIE B list. Depending on the health status, the sanitary conditions and other criteria, molluscs could be exported for human consumption or re-entry in water areas of other countries.

In the B list is included, for bivalve molluscs, Marteiliosis, a disease caused by a parasite which infects different species of shellfish. In Europe, Marteiliosis is caused by *Marteilia refringens* (in *Ostrea edulis* and *M. galloprovincialis*) and *M. maurini* (in *M. galloprovincialis*). *M. refringens* is a protozoan parasite (phylum: Paramyxea), associated with serious and recurrent mortalities in European flat oyster, *O. edulis*. Albania is applying the sanitary and health conditions, according to EU specific legislation for bivalve molluscs, in expectation of export this food in EU countries. For that reason, Albanian coastline has been zoned in some areas exploited for molluscs farming. The sanitary monitoring started in 2004 in some areas: mussels production zone in Butrinti lagoon (2004-2005), *Donax* spp. natural production zone in Karavasta (2004-2005) and *Donax* spp. production zone in Narte-Three Ports-Vjose (2005). This research was carried out in Butrinti lagoon, performing analytical procedures for detection of parasites producing important diseases in molluscs. In this paper are presented the results of sanitary monitoring.

MATERIAL AND METHODS

Sanitary monitoring of mussels in Butrinti lagoon (Figure 1) was carried out in April 2005, suitable month for the detection of *Marteilia* spp. This lagoon was coded BM1 and *M. galloprovincialis* was sampled in three different points, north (BM1N), west (BM1W) and south (BM1S), according to national monitoring plan approved by Ministry of Agriculture, Food and Consumer Protection. From each sampling point, a sample of 150 subjects of *M. galloprovincialis* was taken, as recommended in Manual of Diagnostic Tests for Aquatic Animals (OIE, 2003) for population more than 100,000 (lot size) and disease prevalence in lot $\leq 2\%$ or unknown value.

All collected subjects were kept at a temperature of 4° C, transported to the Ichthyopathology laboratory at the Veterinary Research Institute (Tirana) and analysed by cytological and histological methods. These techniques are approved by Competent Authority and are officially used for sanitary monitoring of molluscs farming.

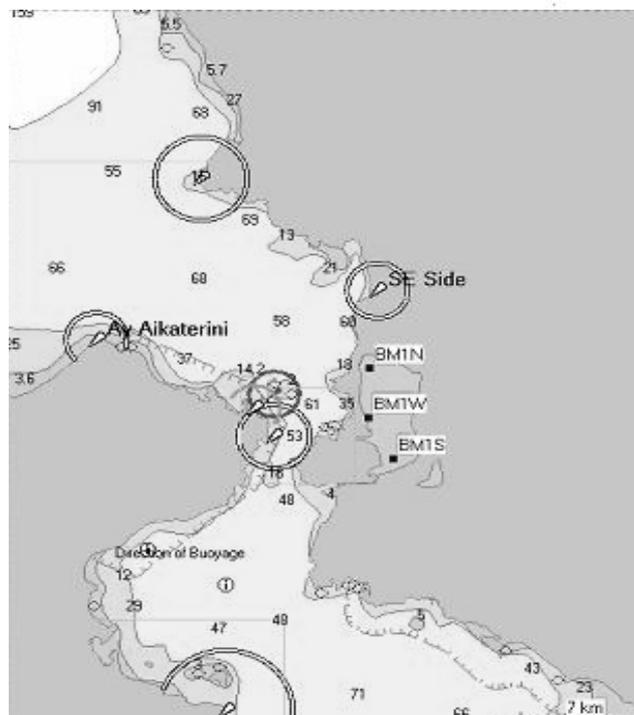


Figure 1 - Map of Burinti lagoon, southern coast of Albania.
Figura 1 – Cartina della laguna di Butrinti, situata nell’Albania meridionale.

For each sample, 100 subjects were washed, taking off deposited matters on shells, and opened without damage organs. Altogether, 300 organisms were analysed by cytological technique (OIE, 2003; 2005): digestive gland imprints on slides were air-dried, fixed in methanol for 2-3 minutes and stained with serial haematoxylin reagents (Hemacolor,

solution 1 and 2, Merck). After this step, all stained slides were examined under light microscopy (oil immersion).

The remaining 50 subjects of each sample were fixed in Carson's solution, in order to prepare histological materials. Standard sections were taken through the digestive gland, to include gills, mantel, gonads and palps. After being embedded in paraffin and sectioned, the slides were stained with haematoxylin-eosin (H&E) and observed under light microscopy. Histological analyses were carried out at Laboratorio Patologia Molluschi of Istituto Zooprofilattico Sperimentale delle Venezie, Udine (Italy).

RESULTS

The cytological investigation, performed on 300 mussels, revealed that 58 mussels were positive for *Marteilia* spp.: 23 coming from the sampling site BM1N, 17 from BM1W and 18 from BM1S. Infection rate was 19.3% of the total number of analysed subjects. By light microscopy examination, different stages of *Marteilia* spp. biological cycle were identified: plasmodium, sporangial primordial and spore primordial. The parasite is characterised by a basophilic cytoplasm and an eosinophilic nucleus.

Histological analyses were performed on 150 subjects. 15 mussels (10% of the total number of analysed subjects) were found positive for the presence of *Marteilia* spp.: 6 coming from BM1N, 6 from BM1W and 3 from BM1S. As we guessed before starting this research, the percentage of infection detected by histological method was lower than the percentage detected by cytological technique.

Sampling point	Cytological method		Histological method	
	N° of positive	%	N° of positive	%
North	23/100	23	6/50	12
West	17/100	17	6/50	12
South	18/100	18	3/50	6
Total	58/300	19.3	15/150	10

Table 1 - Presence of *Marteilia* spp. in *Mytilus galloprovincialis* collected in Butrinti lagoon.
Tabella 1 – Presenza di *Marteilia* spp. in *Mytilus galloprovincialis* campionati presso la laguna di Butrinti.

DISCUSSION AND CONCLUSIONS

Presence of *Marteilia* spp. in mussel farming in Butrinti lagoon was confirmed by this research. Percentage of infection was evaluated by cytological and histological analysis, and resulted respectively in 19.3% and 10% of the total number of examined mussels. A similar research was carried out at the end of June 2004 in Butrinti lagoon; 450 subjects, collected from 4 different sampling areas, were analysed and resulted negative for the presence of

Marteilia spp. It is very important to plan sanitary monitoring, related to mollusc diseases, in suitable months, when diseases are mainly manifested. For that reason, studies for evaluate the presence of *Marteilia* spp. are usually realized in spring and summer. In Italy (Northern Adriatic Sea), outbreaks of Marteiliosis occurred at the end of spring. In mussels, Marteiliosis infection is related to climatic changes, production zone conditions, pollution levels, water movement, geographic conditions and other unknown factors (Robledo & Figueras, 1995). During this study, water temperature was in the range 25-26° C.

Marteilia refringens is a common protozoan parasite which caused mass mortalities in *O. edulis* in several European countries (Grizel *et al.*, 1974; Figueras & Montes, 1988). This situation never occurred in Butrinti lagoon, where mussel seed is not exported from abroad but naturally originates in this place. The infection rate evaluated by this study is comparable with the infection rate of other mussel production areas in the Adriatic Sea (e.g. Gulf of Trieste 1.25% in 2004 and 0.0% in 2005; Venice lagoon 0.01% in 2003; Po Delta River 0.0% in 2004 and 2005). In Croatia, 5% of mussel population was found positive for *Marteilia* spp. (Zrnic *et al.*, 2001).

The analytical methods used (cytological and histological) can not identify the species. Only molecular methods confirmed the difference between *M. refringens* and *M. maurini*. The presence of *M. refringens* and *M. maurini* in *M. edulis* was notified in France, Spain and Italy (Le Roux *et al.*, 2001); these two species of *Marteilia* were also detected in *M. galloprovincialis* in Spain and Italy (Le Roux *et al.*, 2001; Lopes-Flores *et al.*, 2004; Balseiro *et al.*, 2005; Novoa *et al.*, 2005).

Project for revision of EU directive 91/67/EEC related to apply sanitary policy of aquaculture species and their products except the prevention and treatment measures against aquaculture diseases should take into consideration the OIE code.

On the context of EU market, this directive adopts specific sanitary conditions for the placing on market and the import from other non EU countries of different species of aquaculture and their products. EU mission should evaluate if competent authorities of those countries guarantee accomplishing of health conditions and sanitary policy mentioned in EU legislation. Referring to sanitary conditions, collection of bivalve molluscs in natural production zones in Albania should accomplish all conditions requested by EU. Albania government was forced to respect and adopt the sanitary policy and other conditions on aquaculture. This study confirmed that mussels, originated from production zone of Butrinti lagoon, are not allowed to be used for reversion in EU countries because of the presence of *Marteilia* spp.

Mussels harvested in Butrinti lagoon could be exported sending them directly to an approved establishment for bivalve molluscs and imported in respect of directives 91/492/EEC and 91/493/EEC for retail of this product. On the label of packages should be notified "Live bivalve molluscs, destination for immediate human consumption. Not allowed to be kept in tanks and water containers in EU countries" (Decision 2003/804/EEC; Decision 2004/623/EEC).

REFERENCES

Balseiro P., Montes A., Ceschia G., Novoa B. & Figueras A. (2005). Molecular epizootiology of the European *Marteilia* spp., infecting mussels (*Mytilus galloprovincialis* and *M. edulis*) and oysters (*Ostrea edulis*): an update. *In press in Marine Biotechnology*.

Direttiva del Consiglio 91/67/CEE. Norme di polizia sanitaria per la commercializzazione di animali e prodotti di acquicoltura. *Gazzetta ufficiale delle Comunità europee del 19.2.1991*, NL 46: 1-18.

Decisione della Commissione 2003/804/CEE. Condizioni di polizia sanitaria e alla certificazione veterinaria per l'importazione di molluschi, loro uova e gameti, destinati all'accrescimento, all'ingrasso, alla stabulazione o al consumo umano. *Gazzetta ufficiale dell'Unione europea del 20.11.2003*, L 302: 22-33.

Decisione della Commissione 2004/623/CE. Modifica della decisione 2003/804/CE per quanto concerne l'importazione di molluschi vivi destinati al consumo umano. *Gazzetta ufficiale dell'Unione europea del 31.8.2004*, L 280: 26-33.

Figueras A.J. & Montes J. (1988). *Marteilia refringens* Aber disease of oysters. In Fisher, W.S. ed. *Diseases Processes in Marine Bivalve Molluscs. Am. Fisher. Soc., Special Public.*, 18: 38-46.

Grizel H., Comps M., Bonami J.R., Cousserans F., Duthoit J.L. & Le Pennec M.A. (1974). Recherche sur l'agent de la maladie de la glande digestive de *Ostrea edulis* Linné. *Bull. Inst. Peches Marit. Maroc.*, 240 : 7-30.

Kristo R. (2003). Overview of the fishery sector in Albania. *Eurofish*, 5: 58-60.

Le Roux F., Lorenzo G., Peyret P., Audemard C., Figueras A., Vivares C., Gouy M. & Berthe F. (2001). Molecular evidence for the existence of two species of *Marteilia* in Europe. *J. Eukaryot Microbiol.*, 48: 449-454.

Lopez-Flores I., de la Herran R., Garrido-Ramos M.A., Navas J.I., Ruiz-Rejon C. & Ruiz-Rejon M. (2004). The molecular diagnosis of *Marteilia refringens* and differentiation between *Marteilia* strains infecting oysters and mussels based on the rDNA IGS sequence. *Parasitology*, 129: 411-419.

Novoa B., Posada D. & Figueras A. (2005). Polymorphisms in the sequences of *Marteilia* internal transcribed spacer region of the ribosomal RNA genes (ITS-1) in Spain: genetic types are not related with bivalve hosts. *J. Fish Dis.*, 28: 331-338.

OIE (2003). Manual of diagnostic tests for aquatic animals. Part 3, Section 3.1, Chapter 3.1.3 Marteiliosis.

OIE (2005). Aquatic Animal Health Code. Eighth Edition 2005.

Robledo J.A.F. & Figueras A. (1995). The effects of culture-site, depth, season, and stock source on the prevalence of *Marteilia refringens* in cultured mussels (*Mytilus galloprovincialis* Lmk.) from Galicia, Spain. *J. Parasitol.*, 81, 3: 354-363.

Zrncic S., Le Roux F., Oraic D., Sostaric B. & Berthe F.C.J. (2001). First record of *Marteilia* sp. in mussels *Mytilus galloprovincialis* in Croatia. *Dis. Aquat. Org.*, 44: 143-148.