Contamination on the Italian coastline of Pelagros Sanctuary and stranding events of cetaceans during the project

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ITALY
Public Vet Institute under the **Ministry of Health**

- Laboratory for official analysis
- Research Institute
C.Re.Di.Ma.

National Reference Centre for diagnostic activities on dead stranded marine mammals (C.Re.Di.Ma.) recognized by Italian Ministry of Health in January 2015

DECREE 22 October 2014
Coordinate diagnostic activity
Mediterranean Sea is Europe's largest semi-enclosed sea, bathing 22 countries where 507 million people live and welcoming 175 million visitors every year.

Despite international efforts to protect it over the past 30 years, its conditions are deteriorating due to pollution from industries, shipping and the destruction of coastal ecosystems.
VIRUS BACTERIA
PROTOZOA

CLIMATE CHANGE

ACOUSTIC
POLLUTANTS

COLLISIONS WITH
NATANTIS

BY CATCH

AMBIENTAL
POLLUTION

MACRO AND
MICRO PLASTICS

2° Workshop Cetaceans’ contamination in the Pelagos Sanctuary: results and perspectives
Diano Marina (IM), 19th of February 2020
CREDIMA: National Activity

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Pelagos Sanctuary

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Chemical contaminants of anthropogenic origin can compromise humoral and cellulo-mediated immunity and increase susceptibility to infectious diseases (Bossart et al. 2011).

**Biological pollution**
- Virus, Parasites, Bacteria

**Chemical pollution**
- Hydrocarbons, heavy metals, plastics, POPs

**Toxicological stress**

**High susceptibility**
- Large lipid reserves (BLUBBER), accumulation of lipophilic pollutants, which may increase the susceptibility to other stressors

**Chemical and biological pollution interactions**
Ligurian coastline, cause of strandings, 2015-2019 - pathogens

- Aspergillus fumigatus
- Sarcocystis spp
- Toxoplasma gondii
- Listeria monocytogenes
- Salmonella enterica typhimurium monophasic variant 1,4 [5],12:i:-
- Brucella ceti
- Zoonotic agents
- Pathogens indicative of terrestrial contamination
- Cetacean-specific viruses
- Alpha e Gamma Herpesvirus
- Dolphin Morbillivirus

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Ligurian coastline: stranding events investigated vs infectious disease

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severe pathological findings in a high number of cases

able to **compromise the health status** in general, and to cause, in some cases, **immunosuppression** and/or **cerebral impairment**, with disorientation and behavioral changes
OCs levels (HCB, DDTs, PCBs) were estimated at blubber level (Marsili & Focardi, 1997). Results were assessed in terms of immunotoxicity with theoretical reference models (Marsili et al. 2004; Kannan et al. 2000; Jepson et al. 2005).
The cause of death of the 27 cetaceans investigated is as follows:

- **Infectious diseases** (N=19): 70%
- **By catch** (N=4): 8%
- **Other causes (perinatal pathology)** (N=2): 7%
- **Not determined** (N=2): 15%

Correlation between infectious diseases and toxicological stress was observed.
Correlation between infectious diseases and toxicological stress in 27 cetaceans investigated.

- Infectious diseases: 15% (N=19)
- By catch: 7% (N=4)
- Other causes (perinatal pathology): 8% (N=2)
- Not determined: 15% (N=2)

In 11 cetaceans, infectious disease was correlated with toxicological stress (57%).

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Focus on neuropathological findings related to infectious agents (Italian coastline)
inflammatory lesions in 4 cases (DMV/Toxoplasma) (4/10, 40%)
CASE 1

Striped dolphin, juvenile, June 2018, Imperia

a case of by catch secondary to cerebral and systemic toxoplasmosis
brain: *T. gondii* cyst surrounded by glial nodules and mononuclear cell aggregates (IHC)
CASE 2 – 3 – 4

**Striped dolphin, F, calf**
(3 months estimated)
October 2018, Arenzano (GE)

**Striped dolphin, F, adult**
January 2019, Arenzano (GE)

**Striped dolphin, M, adult**
March 2019, Savona

DMV, systemic infection
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specific lesions – malacia and syncytia cells
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EE – EMATOSILINA-EOSINA

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EE vs IHC

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EE vs IHC

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specific lesions – malacia and multinucleated cells
specific lesions – bronco-interstitial pneumonia and syncytia
PHYLOGENETIC ANALYSIS CONDUCTED ON N (176bp) GENE SEQUENCE REVEALED A UNIQUE CLUSTER WITH SEQUENCES RECENTLY IDENTIFIED IN THE ATLANTIC IBERIAN PENINSULA (Bento et al. 2016)
RESEARCH ACTIVITY
Research 16C05

Sarcocystis spp

*Photobacterium damselae damselae*

DMV MoAb
Sarcocystis spp

CNS Stenella coeruleoalba 2011  CNS Stenella coeruleoalba 2012
CNS Stenella coeruleoalba 2017
• MOLECULAR APPROACH

• IHC APPROACH – MoAb and PoAb against S. neurona sent by Dr. Marsh, (Ohio – USA)
<table>
<thead>
<tr>
<th>case</th>
<th>organ</th>
<th>PCR</th>
<th>IHC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I PCR (Miller et al. 2009)</td>
<td>II PCR (Miller et al. 2009)</td>
<td>seq</td>
</tr>
<tr>
<td>2011</td>
<td>Heart (paraffin)</td>
<td>positive</td>
<td>positive</td>
</tr>
<tr>
<td></td>
<td>muscle</td>
<td>positive</td>
<td>positive</td>
</tr>
<tr>
<td></td>
<td>CNS (paraffin)</td>
<td>positive</td>
<td>positive</td>
</tr>
<tr>
<td>2012 (low infested)</td>
<td>Heart (paraffin)</td>
<td>positive</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>muscle</td>
<td>positive</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>CNS</td>
<td>positive</td>
<td>negative</td>
</tr>
<tr>
<td>2017</td>
<td>Heart</td>
<td>positive</td>
<td>positive</td>
</tr>
<tr>
<td></td>
<td>muscle</td>
<td>positive</td>
<td>positive</td>
</tr>
<tr>
<td></td>
<td>CNS</td>
<td>positive</td>
<td>positive</td>
</tr>
</tbody>
</table>
PoAb - 2011 case
Pelagos Sanctuary

- littoral urban development
- harbour activities
- polluted discharges
- heavy industry activities
- waste input through outflow from rivers
QUADRI NEUROPATOLOGICI

Talamo - EE 20 x

Cervelletto - IHC CDV 4 x
21724/19 EE vs IHC

C. OCCIPITALE 10X

C. OCCIPITALE 10X (DMV)
619/19 EE

N. BASE 10X

MESENCEFALO 10X

N. BASE 20X

TALAMO 20X
specific lesions – malacia and multinucleated cells
specific lesions – malacia and syncytia cells
• Mesencephalon 10x
Mesencephalon – IHC toxo 10x
Frontal Cortex – IHC toxo 10x
Pons – neuronophagia 40x

Pons – IHC toxo neuronophagia 40x