PARETIC SYNDROME IN GULLS FROM SOUTHERN PORTUGAL: SEARCHING FOR THE CAUSATIVE AGENT

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INTRODUCTION

Between 2010 and 2019, **2432 gulls (1125 *Larus michahellis* and 974 *L. fuscus*) with paretic syndrome** were received at **RIAS** Wildlife Rehabilitation and Research Centre of Ria Formosa (Olhão, Faro). On admission 333 gulls were dead and 2099 were alive.

Clinical signs: different degrees of ascendant flaccid paresis, weakness, anorexia, diarrhea (flaccid cloacae), dyspnoea and, in some cases, death.

Dead animals were considered as paretic syndrome victims due to the presence of diarrhea, absence of injuries, the date of arrival concurring with a paretic syndrome outbreak and the necropsies findings. Admission numbers have been increasing every year, rocketing in 2019 (see Figures).

Gulls were classified in 5 different levels according to the symptoms severity (see Table). Treatment depended on the severity level and involved basic supportive care: fluid therapy, feeding assistance and treatment of secondary conditions until full autonomy was recovered.

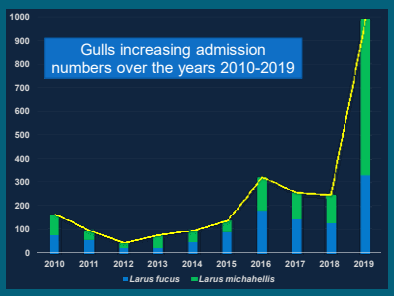
What´s the posible cause?

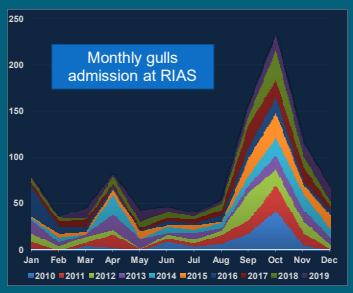
Several biotic contaminants could be the potential cause of this syndrome: marine biotoxins, *Clostridium botulinum*, cyanotoxins and virus. Other factors (starvation, abiotic contaminants, etc) could also contribute to the gulls death. The veterinary from RIAS contacted several research Institutes seeking for collaborations that could help ascertain the reason of the continuous morbidity and mortality episodes. Pasteur Institute (Paris) and the Spanish Oceanographic Institute (IEO Vigo) agreed to perform toxin analyses.

Level	Symptoms	Admission procedures	*Larus michahellis* and *L. fuscus*
1	•Unable to fly •Weakness & dropped wings •Able to walk & autonomously feed •Diarrhea	•Subcutaneous fluid therapy • Solid palatable food offered •Water *ad libitum*	
2	•Half-standing •Mild anorexia or feeding autonomously •Diarrhea	•Subcutaneous fluid therapy •Solid palatable food offered. If not accepted tube feeding •Water *ad libitum*	
3	•Hind limbs paralysis •Mild dyspnoea •Partial or complete third eyelid paralysis •Anorexia •Diarrhea	•Subcutaneous fluid therapy •Tube feeding until solid food accepted •Water *ad libitum*	
4	•Severe dyspnoea •Complete third eyelid paralysis •Legs & neck paralysis •Anorexia •Diarrhea	•Subcutaneous and intravenous fluid therapy •Repeat fluid therapy for 24 or 48 hours until tube feeding is safe	
5	•Irreversible & severe full body paralysis •Severe inability to breath •Diarrhea •Quick death	•Subcutaneous and intravenous fluid therapy •Repeat fluid therapy for 24 or 48 hours until tube feeding is safe	

OBJECTIVES

The **aim** of this preliminary study was to evaluate if Amnesic Shellfish Toxins (Domoic acid, (DA)), Paralytic Shellfish Toxins (PSTs) or botulinum toxins could be the cause of the gulls paretic syndrome. This presentation compiles the results of the *Clostridium botulinum* and marine biotoxins analysis conducted at the French National Reference Centre for anaerobic bacteria and botulism, Pasteur Institute (Paris) and the Vigo Centre of the Spanish Oceanographic Institute, respectively.





METHODS & RESULTS

Paralytic Shellfish Toxins analyses

• Gulls samples: ten kidneys & contents from one cloacae.

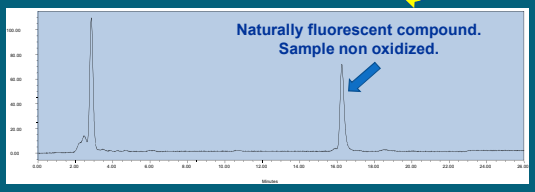
• Drawbacks: very small sample size, difficult homogenization, extraction had to be scaled down.

• Double extraction with 0.1M HCl. Sample deproteination with trichloroacetic acid.

• Analyses by Liquid Chromatography with Postcolumn Derivatization & Fluorescence Detection.

• Samples re-run without oxidation: checking for naturally fluorescent compounds needed

GTX1, GTX2, GTX3, GTX4, dcGTX2, dcGTX3, GTX5, dcSTX, STX were not detected in any of the samples tested



Domoic acid analyses

•Samples: twenty three gull samples: ten livers, ten intestines and three cloacae contents.

• Drawbacks: small sample size, difficult homogenization (specially for intestines, hard to cut by Ultraturrax, pre-cutting needed).

• Single dispersive extraction: with MEOH 50%

• Liquid Chromatography coupled to High Resolution Mass Spectrometry.

DA was not detected in any of the samples tested

Clostridium botulinum analyses

• Samples: livers & intestines from 5 gulls. Pooled in two groups according to the tissue.

• Enrichment cultures of tissues in fortified cooked meat medium at 37°C and 15°C under anaerobic conditions. *C. botulinum* was isolated on agar selective medium.

• DNA extraction from samples.

• *C. botulinum* was investigated by targeted Real Time Polymerase Chain Reaction (PCR) on neurotoxin genes.

•Presence of botulinum toxins was confirmed by a lethality test on mice (mouse bioassay). Mice were intraperitoneally injected with filtered supernatant of the culture.

Samples were positive for *C. Botulimum* types C/D

CONCLUSIONS

• Domoic acid and Paralytic Shellfish Toxins do not seem to be the cause of the gulls paretic syndrome.

• **Samples tested point to *Clostridium botulinum* as the possible cause of the gulls paretic syndrome, or at least one of the causes. This bacterium is considered as a frequent cause of massive mortality events in seabirds. The symptoms caused in seabirds are very similar to the ones produced by PSTs.**

• **Further studies are needed to evaluate the source of *C. botulinum* contamination.**

• Additional studies are planned in other Institutions to evaluate the potential implication of Cyanotoxins and Tetrodotoxins in the toxic episodes.

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