INTRODUCTION

Between 2010 and 2019, 3432 gulls (1125 Larus michahellis and 974 L. fuscus) with paralytic 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, on different degrees of ascendant, included paretic: weakness, anorexia, diarrhea (flaccid cloaca), dyspnoea and, in some cases, death.

Dead animals were considered as paralytic syndrome victims due to the presence of diarrhea, absence of injuries, the date of arrival coincuring with a paralytic 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 possible cause?

Several basic 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 Institutions seeking for collaborations that could help ascertain the reason of the continuous morbidity and mortality episodes. Pasteur Institute (Paris) and the Spanish Oceanographic Institute (EOI Vigo) agreed to perform toxin analyses.

OBJECTIVES

The aim of this preliminary study was to evaluate if Amnesic Shellfish Toxins (Domoic acid, DA), Paralytic Shellfish Toxins (PSTs) or botulism toxins could be the cause of the gulls paralytic syndrome. This presentation compiles the results of the Clostridium botulinum and marine biotoxin 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 cloaca.
- 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 Dextranose Fluorometric 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.

Clostridium botulinum analyses

- Samples: twenty three gull samples: ten livers, ten intestines and three cloaca contents.
- Drawbacks: small sample size, difficult homogenization (speciality 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.

Domoic acid analyses

- Samples: three gull samples: ten livers, ten intestines and three cloaca contents.
- Drawbacks: small sample size.
- Analyses by Liquid Chromatography with Postcolumn Dextranose Fluorometric Detection.
- DNA extraction from samples.
- Polymerase Chain Reaction (PCR) on neurotoxin genes.
- Enrichment cultures of tissues in fortified cooked meat medium at 37°C under anaerobic conditions.
- C. botulinum was isolated on agar selective medium.
- DA extraction from samples.

Samples were positive for C. Botulinum types C/D.

CONCLUSIONS

- Domoic acid and Paralytic Shellfish Toxins do not seem to be the cause of the gulls paralytic syndrome.
- Samples tested point to Clostridium botulinum as the possible cause of the gulls paralytic syndrome, 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.

REFERENCES


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