THE FIRST CASE OF TYPE F BOTULISM, IN PORTUGAL

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Introduction:

The first case in Portugal of type F botulism was identified in July 2016. Type F botulinum toxin (BoNT/F) was detected and a BoNT/F producing strain was isolated in the stool of a patient with clinical signs of botulism.

Worldwide, few cases of type F botulism have been reported in adults [1]. Most cases of botulism described in humans have been caused by toxins of type A, B or E. The microorganisms that have been identified as producers of toxin F are *C. botulinum* and *C. baratii* [1]. Ingestion of contaminated food or, more rarely, wound infection, toxin production in the intestinal tract if immunologically immature (infant botulism) or intestinal colonization in the adult, can produce the clinical syndrome of botulism [1].

Case description:

A 53-year-old man was admitted to the Emergency Department of the S. Bernardo Hospital in Setúbal, with clinical symptoms of botulism that included blurred vision, diplopia, ptosis, dysarthria, dysphagia, dry mouth and non-reactive bilateral mydriasis. The patient reported nausea, abdominal distress and vomiting, after dinner on the previous day. He was hospitalized with the possible diagnosis of botulism versus atypical Guillain-Barré syndrome. Subsequently, he presented progressive respiratory distress, urinary retention and flaccid paraparesis, and was transferred to the intensive care unit. A serum sample and a stool sample resulting from a micro enema were sent to the National Institute of Health Doutor Ricardo Jorge, I.P. (INSA). Although botulinum toxin (A, B, E) was administered, the patient required ventilatory support for 8 days. The patient was discharged after 30 days and three months later was asymptomatic.

Epidemiological investigation:

The epidemiological investigation was performed by the Local and Regional Health Authority. An inquiry was carried out to identify suspect foods consumed within 72 hours prior to the start of complaints [2] and the members of the patient's family were questioned. A home-prepared tuna salad was identified as the suspect meal, which was consumed only by the patient. In the absence of any leftovers or traceability of canned batch of the tuna ingested, two intact canned tuna packages of two different batches, of the same brand as the one ingested by the patient, were sent to INSA as well as fresh onions and tomatoes and pepper, identical to those used in the preparation of the salad and also coffee capsules from the same batch of the one ingested after the meal. Other foods ingested within 72 hours before the symptoms onset, which were not traceable, cannot be excluded as suspects, although were shared with other people who did not complain.

Laboratory investigation:

Method	Serum	Stool	Foodstuffs
 Bioassay	✓	✓	✓
 Molecular		✓	✓
 Cultural		✓	✓

Serum and stool samples were tested for the presence of botulinum toxin by bioassay in mice [3].

After enrichment culture in Tryptone Peptone Glucose Yeast Extract Broth, at 30 ± 1°C and/or Cooked Meat Medium, at 37 ± 1°C of the stools and the food samples, the detection of botulinum toxin-producing clostridia by multiplex-PCR and the strain isolation by cultural method was carried out [4,5]. It was also performed the bioassay toxin detection for the two canned tuna samples [3].

Conclusion:

Some of the few cases of BoNT/F reported in adults were due to intestinal colonization and others were food-borne [1]. The fact that the patient had gastrointestinal disturbances leads us to suspect of food poisoning. As in most of the reported cases of type F botulism, the suspicion attributed to a particular foodstuff was not identified with strong evidence [1].

Results:

Specimen source		Mice assay toxin detection		PCR - BoNT producing clostridia	Strain isolation
		Direct	Toxin type identified		
Serum		Signs suggestive of botulism and death after 3 days	Inconclusive	-	-
Stool	Extract	Signs suggestive of botulismand and death	BoNT/F	-	-
	Enrichment culture	Signs suggestive of botulism and death	BoNT/F	BoNT/F positive	Yes *C. Botulinum*
C. botulinum/ Strain isolated from stool	Enrichment culture	Signs suggestive of botulism and death	BoNT/F	BoNT/F positive	-
Tuna can 1	Extract	Negative	-	Negative	-
	Enrichment culture	Negative	-	Negative	No
Tuna can 2	Extract	Negative	-	Negative	-
	Enrichment culture	Negative		Negative	No
Tomato	Enrichment culture	Negative	-	Negative	No
Onion	Enrichment culture	Negative	-	Negative	No
Pepper	Enrichment culture	Negative	-	Negative	No
Coffee	Enrichment culture	Negative	-	Negative	No

Inoculation of 1 ml of serum in Balb/c caused typical botulism symptoms sequence including ruffled fur, laboured abdominal breathing, wasp shape, weakness of limbs progressing to total paralysis, gasping for breath and death due to respiratory failure after 3 days. After toxins neutralization, none of the mice showed the typical symptom sequence of botulism, so the result was inconclusive.

The presence of type F botulinum toxin and also genes of clostridia encoding BoNT/F were detected in the stool sample. A *C. botulinum* BoNT/F producing strain was isolated.

The analysis of canned tuna was negative for toxin with bioassay [2] and BoNT-producing clostridia were not detected (with multiplex-PCR and cultural method) in the foodstuffs analyzed.

References:

[1] European Centre for Disease Prevention and Control. Scientific advice on type F botulism. Stockholm: ECDC, 2013. [2] Heymann DL. Control of communicable diseases. 20th ed. Washington, DC: American Public Health Association Press, 2013. [3] Centres for Disease Control. Clostridium botulinum Monovalent and Polyvalent Antitoxins. Atlanta, Georgia: CDC, 1987. [4] Austin JW, Sanders G. Detection of Clostridium botulinum and its toxins in suspect foods and clinical specimens. In: Health Canada Compendium of Analytical Methods. HPB Methods for the Microbiological Analysis of Foods MFHPB-16, vol. 2. Ontario: Health Canada, 2009, pp. 1–9. [5] De Medici D, Anniballi F, Wyatt GM, et al. Multiplex PCR for detection of botulinum neurotoxin-producing clostridia in clinical, food, and environmental samples. Appl Environ Microbiol. 2009;75(20):6457-61.