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ORIGINAL

Protophormia terraenovae. A new allergenic species in amateur fishermen of Caceres, Spain

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Allergy;
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Fish bait;
Fly larvae.

Abstract
Background: Asticot maggot (Blowfly, *Calliphoridae* family) is the most important live bait used for angling in our country. Prevalence of allergy to live fish bait in occupationally exposed workers has been described. The purpose of this study was to determine the prevalence of asticot allergy in amateur fishermen and the identification of marketed asticot species in Cáceres, Spain.
Materials and Methods: Seventy-two randomised selected patients (Angler's Society of Cáceres) completed a questionnaire about fishing habits and allergic symptoms related with live bait handling. Skin prick test (SPT) with local asticot and common earthworm extracts were performed. Serum IgE levels to imported species (*Protophormia terraenovae, Calliphora vomitoria, Lucilia sericata, Lumbricus terrestris*) were measured. Local asticot and common earthworm samples were obtained for taxonomic identification. Data were analysed using the SPSS 12.0 software.
Results: Five patients (7%) reported allergic symptoms caused by asticot maggots. All of them were positive for SPT to asticot and specific IgE to *P. terraenovae*. Sensitisation to *P. terraenovae* was found in 40 patients (58.8%). No associated factors for asticot allergy were observed. Larvae and adult flies of local asticot samples were identified as *P. terraenovae*.
Conclusions: Commercially available asticot, in Cáceres, is composed by *P. terraenovae larvae* (*Diptera. Calliphoridae*). A 7% prevalence of *P. terraenovae* allergy in amateur fishermen of Cáceres was obtained. The allergenic potential of *P. terraenovae* seems to be greater than that of other blowflies and *L. terrestris*. The SPT with *P. terraenovae* extract is a very sensitive and specific technique in the diagnosis of live bait allergy in fishermen.

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Introduction

Freshwater sport fishing is one of the most popular pastimes in Cáceres, Spain. The asticot is the most commonly used live bait in the area, having replaced more traditional baits, such as the earthworm, thanks to its great avidity for all types of fish. The name "asticot" is used by French companies to sell fly larvae from the *Calliphoridae* family as live fishing bait. Within the asticot range, there are various fly species that are practically indistinguishable as larvae. There are over 1000 Calliphoridae species in existence worldwide, with less than 50 known species in Spain. *P. terraenovae* (of the *Calliphoridae* family) is a species that prefers low temperatures for its offspring and is capable of surviving extreme climatic conditions. This explains its abundance, especially in cold regions and in the higher-altitude areas of milder regions. In Spain, only a few wild *P. terraenovae* specimens have been proven to exist in the Aragonese Pyrenees[1].

In 1982, Stockley et al[2] described the first case of hypersensitivity to fly larva of the *Calliphoridae* family in a fisherman with contact urticaria and late asthmatic responses related to the use of this bait. Since then, in most of the cases described of allergic reactions to fly larvae in exposed fishermen or workers, the species involved primarily pertain to the *Calliphora spp* and *Lucilia spp* genera (*Calliphoridae* family). The studies conducted by Siracusa et al. demonstrate the allergenic importance of *Lucilia caesar*[3] and *Calliphora vomitoria*[4] in comparison with other live bait. There are no published cases of allergies to *P. terraenovae* in international literature, except for one case described in a Spanish communication[5].

Considering how often live bait is used and its ability to provoke allergic reactions, there is a lack of studies which assess the allergenic potential of this live bait in fishing enthusiasts. Therefore, we present a descriptive study on allergic reactions caused by fishing bait in a group of fishermen in Cáceres. The objectives of this study are to identify the bait involved, the frequency of the presentation of allergic reactions to them, and to analyse the possible risk factors for their development.

The difficulties in diagnosing the allergic pathology caused by this bait are primarily based in the problems of selecting and identifying the raw materials and in the lack of biologically standardised extracts. The aim of this study is to carry out a taxonomic identification of the dipteran sold as asticot in the fishing equipment stores in Cáceres. In this way, specific extracts can be made of the specific species to which the fishermen of our region are exposed.

Materials and Methods

Taxonomic Identification

Various samples of asticot and common earthworms were gathered from three fishing equipment stores in the city of Cáceres. Imported samples (Verminiere de L'ouest. Tremblay, France) were also obtained from the asticot range, sold and labelled with the following taxonomic identification: "l'asticot (*P. terraenovae*)", "le gozzer (*C. vomitoria*)" and "le pinkie (*L. caesar*)", as well as *L. terrestris* (common earthworm). Once these samples were collected, they were sent to the Parasitology Unit of the Faculty of Veterinary Medicine at the University of Extremadura, for their taxonomic identification. Fifty third-instar larvae were analysed from each of the samples. A first provisional classification was established by following the guidelines of Smith (1986)[6]. In order to ensure that the dipteran species were correctly identified, their biological cycle was completely carried out until adult flies were obtained. These were then classified following the guidelines of González-Mora & Peris[1], González-Mora[7], and Peris & González-Mora[8].

Diagnostic extracts

Local and commercially available asticot and common earthworm species were extracted in phosphate-buffered saline (PBS) 50% w/v at 4-8 °C. After it had been stirred for 2 h (1000 rpm), the solution was centrifuged at 10.000 g for 30 min. The resulting supernatant was then passed through Watman filters. This filtration was dialyzed in a dialysis membrane (Visking 7000 Da) for 12 hours at 4 °C. The extract resulting from the dialysis was then filtered in a vacuum through filters with a Millipore GS depth of 0.45 μm. It was then frozen at −20 °C in order to undergo a lyophilisation process. The protein concentration of the final extract was determined by Bradford assay as previously described[9]. The extracts were reconstituted at a 5 mg/ml concentration at the moment of their use for in vivo and in vitro tests.

Subjects and diagnostic procedures

The study was presented to the Ethics Committee of the hospital Complejo Hospitalario de Cáceres with a favourable outcome. Seventy-two randomly-selected patients (members of the Cáceres Fishing Association) agreed to participate in the study. All the patients filled in a questionnaire based on the ECRHS[10] and ISAAC[11] epidemiological questionnaires with a series of modifications that included questions regarding fishing habits and the possible presence of symptoms (skin, nasal, ocular, respiratory) related to the use of live bait. That same day, blood samples were taken in order to obtain the serum that was frozen at −20 °C until its use in the specific IgE determinations. After the samples were taken, skin prick tests (SPT) were carried out with the asticot and local earthworm extracts in all the patients. SPT were performed with the allergy pricker lancet (Dome-Hollister-Stier) as described elsewhere[12]. Histamine phosphate (10 mg/ml) served as positive control and NaCl (0.9%) as negative control. The tests were read after 20 minutes. A reaction was considered to be positive if the wheal size was at least 3 mm diameter larger than negative control. Ten atopic and ten non-atopic controls were tested for SPT. All the patients underwent a prick test with a battery of conventional inhalant allergens (Laboratorios Diater, Spain). Atopics were defined as having a positive clinical history and a SPT reaction more than 3 mm to at least one common allergen.

Specific IgE levels to imported samples of asticot and *L. terrestris*, as determined by ELISA method, were measured at the end of patient recruitment. Briefly, protein extracts (250 μg/ml) were bound to 96-well polystyrene microplates (Costar, Cambridge, Mass.) by overnight incuba-