



Fish allergy risk derived from ambiguous vernacular fish names: Forensic DNA-based detection in Greek markets

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ABSTRACT

Some fish species, like hakes, are amongst the most allergenic seafood species and represent a high proportion of food allergies, particularly in children. Seafood mislabelling or incomplete labelling may enhance inadvertent consumption of allergenic species. The problem can be accentuated in countries where vernacular names do not distinguish fish species, like in Greece, where hake, cod and other Gadoids are generically named with the same Greek word *Bakaliarios*. The purpose of the study was to detect and quantify allergenic fish species contained in commercial seafood labelled with generic and unspecific names. Commercial samples were obtained at random from Greek fish markets and fish species were identified based on PCR fragments of the 5S rDNA. Almost 85% of the analysed products contained highly allergenic hake or grenadier species and only 15% contained less allergenic species like cod and haddock. We conclude that commercial fish markets are potential hotspots of allergy risk, and suggest labelling containing species names and consumer education for reading labels as preventive measures.

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1. Introduction

Food-allergic consumers are advised to exclude allergenic foods from their diet. A considerable proportion of accidental exposures are attributed to inappropriate labelling (Sheth et al., 2010). Therefore, identification of potential hotspots of mislabelling is crucial for targeting specific sectors, where application of existing laws, or development of new amendments if necessary, needs to be improved.

In Greece, the prevalence of food allergy is about 2.5–4% of Greek adults and 6–8% of children and the rate of prevalence indicate increasing trends (Papageorgiou, 2002; Pitsilidis, 2010). Thus, at least 400,000 to 500,000 Greeks are suffering from food allergies. Fish allergy represents the third cause of food allergies in children, after milk and eggs (Pascual et al., 2008), and is present in >20% patients with a diagnosis of food hypersensitivity in coastal countries as diverse as South Africa (Zinn, Lopata, Visser, & Potter, 1997), Japan (Aranishi & Okimoto, 2004) and Spain (Pascual, Martín Esteban, & Crespo, 1992). All fish species are not equally allergenic. Amongst the species commonly consumed in Europe, hake is considered the one with the strongest allergic response (Pascual et al., 1992) followed by cod, salmon and pollack whereas other species like tuna and halibut

are less allergenic (e.g. Koyama et al., 2006; Van Do, Elsayed, Florvaag, Hordvik, & Endresen, 2005). In the absence of fraud, if fish products are labelled with the accurate scientific species name, consumers can be informed about the species contained in a commercial package and can eat seafood safely.

However, mislabelling of commercial fish can reach high levels in some seafood products like surimi (Pepe et al., 2007), and is a potential source of accidental exposure to allergenic species. More than 20% mislabelling has been detected in marketed lots of hake (Machado-Schiaffino, Martinez, & Garcia-Vazquez, 2008), which is the most allergenic fish in Spain. Therefore one of the potential hotspots of allergy risk is the hake market.

The risks of allergy derived from hake mislabelling would depend on fish consumption and market presentations in each country. In a Mediterranean country with high rate of fish consumption, Greece (Arvaniti, Panagiotakos, Pitsavos, Zampelas, & Stefanadis, 2006), Gadoids are generically named with the Greek word *μπακαλιάρος* (“bakaliarios”). The word is used for naming indistinctly different species like hakes, cod, haddock and others which are not native of Greek waters but are imported from other countries and they are labelled with the generic Greek common name. For a fish allergic consumer, the risk of allergy associated to “bakaliarios” will depend on the species present under that label since not all the Gadoids are equally allergenic; for example, whiting, hake and grenadier are more allergenic than cod based on their IgE-binding activity (Koyama et al., 2006).

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The aim of this study was to detect the levels of highly allergenic mislabelled species in Greek market consumed by inapprehensive consumers. The target was imported fish species in local Greek markets. Forensic DNA analyses of imported commercial fish products labelled as μπακαλιάρος were carried out. We employed the 5S rDNA as species-specific marker (Perez & Garcia-Vazquez, 2004).

2. Material and methods

2.1. Samples

A number of control species were used for checking our genetic results against reference samples. These are species of white-flesh fish that look like hake when sliced or processed. Reference samples for gadoid species (e.g. Atlantic cod *Gadus morhua*, whiting *Merlangius merlangus*, haddock *Melanogrammus aeglefinus*, common ling *Molva molva* and pollack *Pollachius virens*) were provided by the Spanish Institute of Oceanography (Instituto Español de Oceanografía, IEO). Thirty individuals from different distribution areas were analysed. Reference samples for hake species imported to Greece (*Merluccius australis*, *M. bilinearis*, *M. capensis*, *M. gayi*, *M. hubbsi*, *M. paradoxus* and *M. senegalensis*, and the long-tail hake or Grenadier *Macruronus magellanicus*) were taken from Perez and Garcia-Vazquez (2004).

A total of 33 commercial lots labelled with the Greek name “μπακαλιάρος” were sampled from Greek supermarkets, directly purchased at random from eleven different stores (three lots per store) located in the region of Thessaloniki, with more than 1,000,000 inhabitants. Three pieces were analysed per lot. We did not consider autochthonous species that can be caught in Greek waters.

2.2. Forensic analysis

DNA was extracted for PCR amplification of the 5S rRNA gene following the Chelex-based protocol described in Perez and Garcia-Vazquez (2004). PCR amplification of the 5S rRNA was performed using the primers described by Pendas, Moran, Martinez, and Garcia-Vazquez (1995). Amplifications were carried out using the GeneAmp PCR system 2400 by Perkin Elmer Cetus on samples containing approximately 50 ng of DNA sample, 10 mM Tris–HCl pH 8.8, 1.5 mM MgCl₂, 50 mM KCl, 0.1% Triton X-100, 20 pmol of each primer, 1 U Dynazyme II DNA polymerase (Finnzymes) and 250 μM dNTP in a final volume of 20 μl. PCR was performed with an initial denaturing step (5 min at 95 °C) followed by 30 cycles consisting of: denaturing 20 s at 95 °C; 20 s annealing at 65 °C; extension at 72 °C for 20 s; and a final extension at 72 °C for 7 min.

The fragment sizes of the PCR products were determined employing the following protocol: 1 μl of PCR products was mixed with 10 μl of deionized formamide and 0.3 μl of GeneScan-500 LIZ Size Standard (Applied Biosystems). Fluorescent fragment detection was performed by capillary electrophoresis in a 3100 Genetic Analyzer (Applied Biosystems) with a 36 cm capillary and POP 4 polymer. Electrophoresis conditions were those given as default by the manufacturer. Fragment sizes were established using the GeneScan 3.7 Analysis Software (Applied Biosystems).

3. Results

Fragment sizes obtained after PCR amplification of the 5S rDNA genes of reference samples were different for all the considered species (Table 1). Intraspecific variation was not found in any case.

The commercial products analysed yielded positive PCR amplification of the genetic marker employed, regardless of their different presentation. Some were frozen, presented as whole pieces or as slices or fillets, and four lots were pre-cooked finger fish. Successful DNA extraction and PCR amplification with the primers employed for the 5S rDNA was obtained from all of them. The commercial lots, all

Table 1

Fragment sizes obtained after PCR amplification of the 5S rDNA genes employing the primers designed by Pendas et al. (1995) in the reference samples. In parentheses, secondary amplification bands (less intensely amplified).

Species	Common name	Fragment sizes
<i>Gadus morhua</i>	Atlantic cod	538 (+ 146)
<i>Melanogrammus aeglefinus</i>	Haddock	158
<i>Molva molva</i>	Common ling	158 + 270
<i>Pollachius virens</i>	Pollack	530
<i>Merlangius merlangus</i>	Whiting	568
<i>Macruronus magellanicus</i>	Long-tail hake or Grenadier	272
<i>Merluccius australis</i>	Austral hake	400
<i>Merluccius bilinearis</i>	Silver hake	222 (+ 661 + 759)
<i>Merluccius capensis</i>	Cape hake	371
<i>Merluccius gayi</i>	Chilean hake	386 (+ 393)
<i>Merluccius hubbsi</i>	Argentinean hake	241 (+ 660)
<i>Merluccius paradoxus</i>	Deep Cape hake	371 + 492
<i>Merluccius senegalensis</i>	Senegalese hake	365

labelled with identical names (μπακαλιάρος, “bakaliaros”), contained seven different species (Table 2). The three pieces analysed of each lot corresponded to the same species, and exhibited fragment sizes (Fig. 1) that unambiguously corresponded to the reference samples. The species were identified accordingly as Argentinean hake *Merluccius hubbsi*, Senegalese hake *M. senegalensis*, Chilean hake *M. gayi*, Deep Cape hake *M. paradoxus*, Atlantic cod *G. morhua*, haddock *M. aeglefinus* and long-tail hake or Grenadier *M. magellanicus* (13, 10, 3, 1, 2, 2 and 2 lots respectively). In total 28 lots (84.8%) contained hake (true hake *Merluccius*, long-tail hake or grenadier *Macruronus*), and only 15.2% lots contained other less allergenic marine fish species.

4. Discussion

Although based on a somewhat limited sample size, this survey identifies the Greek commercial market of imported fish as a potential source of allergy risk derived from inadvertent consumption of hake, since hake species constitute one of the most allergenic fish groups detected so far (Koyama et al., 2006; Pascual et al., 1992). Grenadiers are also highly allergenic (Koyama et al., 2006). Other species labelled under the same generic name like the Atlantic cod *G. morhua* and the haddock *M. aeglefinus* are also allergenic, but at a lower level (Koyama et al., 2006).

Clear and consistent labelling of food allergens combined with increased consumer education is necessary to improve consumer confidence and compliance and to reduce accidental exposures (Sheth et al., 2010). Recent studies revealed that food allergic Greek consumers do not spend too much time on reading labels, except when buying products which they had not bought before (Cornelisse-Vermaat, Voordouw, Yiakoumaki, Theodoridis, & Frewer, 2008). Pan-European labelling policies with some national differences are feasible (Cornelisse-Vermaat et al., 2008) and therefore a recommendation could be the use

Table 2

5S rRNA fragment sizes obtained for products labelled as “bakaliaros” in Greece, and putative species genetically identified. N = number of lots sampled.

Product	5S fragments	Putative species	N
Filet	241	Argentinean hake (<i>Merluccius hubbsi</i>)	5
Slice	241	Argentinean hake (<i>M. hubbsi</i>)	4
Whole piece	241	Argentinean hake (<i>M. hubbsi</i>)	4
Filet	365	Senegalese hake (<i>M. senegalensis</i>)	1
Slice	365	Senegalese hake (<i>M. senegalensis</i>)	1
Whole piece	365	Senegalese hake (<i>M. senegalensis</i>)	8
Slice	386	Chilean hake (<i>M. gayi</i>)	1
Whole piece	386	Chilean hake (<i>M. gayi</i>)	2
Whole piece	371, 492	Deep Cape hake (<i>M. paradoxus</i>)	1
Filet	538	Atlantic cod (<i>Gadus morhua</i>)	2
Precooked	158	Haddock (<i>Melanogrammus aeglefinus</i>)	2
Precooked	272	Long-tail hake (<i>Macruronus magellanicus</i>)	2

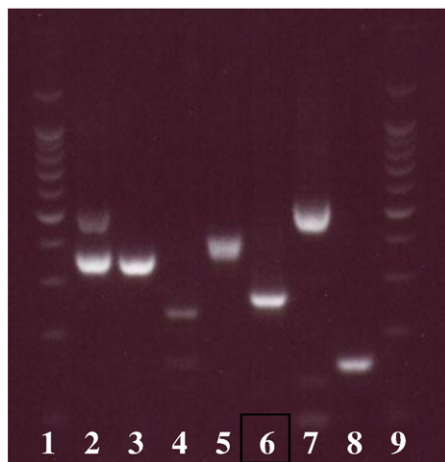


Fig. 1. Agarose gel (2.5%) stained with ethidium bromide showing amplification fragments obtained for the 5S rDNA from Greek commercial samples labelled as “bakaliaros” and corresponding to different species. From left to right: 1 and 9: DNA size marker (100 nucleotides of difference between consecutive fragments); 2: Deep Cape hake *Merluccius paradoxus*; 3: Senegalese hake *Merluccius senegalensis*; 4: Argentinean hake *Merluccius hubbsi*; 5: Chilean hake *Merluccius gayi*; 6: grenadier *Macruronus magellanicus*; 7: Atlantic cod *Gadus morhua*; and 8: haddock *Melanogrammus aeglefinus*.

of clear big labels with the specific name in Latin, particularly important for a local consumer that does not differentiate cod and hake by the common name.

Finally, on the technical side of the study it should be said that other DNA-based markers (Rasmussen & Morrissey, 2009) could be employed for identifying all the products analysed in this study. The main advantage of the method employed here is that the 5S rDNA PCR fragment sizes found in this work can be perfectly identified in acrylamide or in agarose gels, which are extremely cheap (Perez & Garcia-Vazquez, 2004), but there are not reference databases with 5SrDNA fragment sizes of many species. In contrast, sequences of different genes exist for many fish species in public databases like GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>, accessed June 2010) and more than 54,000 DNA sequences are stored in the FISH-Barcode database (Hebert, Cywinska, Ball, & deWaard, 2003; <http://www.fishbol.org/>, accessed June 2010) providing references for more than 7000 species, including most commercial species distributed worldwide. In a near future the choice of DNA markers for fish forensic studies will depend more on laboratory budgets than on availability of reference samples.

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