Profile of Sensitization to Related Animal Proteins (Crocodile, Frog, and Chicken) Among Fish-Allergic Patients

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J Investig Allergol Clin Immunol 2022; Vol. 32(1): 50-52
doi: 10.18176/jiaci.0705

Key words: Fish allergy. Crocodile. Frog. Chicken. Cross-reactivity.

Palabras clave: Alergia a pescado. Cocodrilo. Rana. Pollo. Reactividad cruzada.

Fish allergy is one of the most common food allergies, particularly in children [1,2]. Parvalbumins are major allergens present in the white muscle of lower vertebrates, and cod parvalbumin (Gad c 1) was the first parvalbumin studied [3]. Other fish allergens include tropomyosin, collagen, aldehyde phosphate dehydrogenase, enolase, and aldolase [1,4]. Cross-reactivity between fish parvalbumins, which is due to their high amino acid sequence identity, is responsible for allergy to various fish species [5]. However, some patients have selective specific IgE antibody (sIgE) reactivity to single parvalbumins and develop symptoms to a particular species [6-8].

Cross-reactivity between fish and chicken due to parvalbumin and other allergens has been reported [9]. Published findings describe allergy to fish and other animals such as frog [10], chicken [9,11], and crocodile [12], as well as to crocodile and chicken [13]. These allergies result from cross-reactivity between parvalbumins. Two crocodile parvalbumins (nCro p 1, Cro p 2) have been identified (WHO/IUIS; www.allergen.org).

We aimed to study the pattern of IgE reactivity in extracts from crocodile, frog, and chicken using sera from fish-allergic patients.

Patients with a clear history of fish allergy, positive skin prick test (SPT) results, and/or positive sIgE findings for fish extract were evaluated. Oral challenge was performed in cases of a negative SPT and/or sIgE result [14].

Crocodile, frog, cod, and chicken were purchased fresh, and extracts were prepared from raw and cooked foods as described elsewhere [9]. SPT was performed with raw and boiled crocodile extract (10 mg/mL) and 7 fish extracts (Roxall). Prick-by-prick tests were performed with raw and boiled crocodile, frog, and chicken meats. Nonatopic patients were included as negative controls.

We included 27 patients with fish allergy (median [IQR] age, 8 years [5-17; range, 2-46]), of whom 18 were male. Hake

Table. Skin Test and Specific IgE to Crocodile, Frog, and Chicken Extracts

	Crocodile					Frog			Chicken		
	Prick test		Prick-prick		EAST, kU_A/L[a]	Prick-prick		EAST, kU_A/L	Prick-prick		CAP, kU_A/L[a]
%	RCrme	BCrme	RCrPP	BCrPP	Cr	RFPP	BFPP	F	RChPP	BChPP	Ch
Positive	78.2	66.6	80.7	61.5	85.2	69.5	59	77.7	78.2	40.9	64
Negative	21.8	33.4	19.3	38.5	14.8	30.5	41	22.3	21.8	59.1	36

Abbreviations: BChPP, boiled chicken prick-prick; BCrme, boiled crocodile meat extract; BCrPP, boiled crocodile prick-prick; BFPP, boiled frog prick-prick; Ch, chicken; Cr, crocodile; EAST, enzyme allergosorbent test; F, frog; RCrme, raw crocodile meat extract; RChPP, raw chicken prick-prick; RCrPP, raw crocodile prick-prick; RFPP, raw frog prick-prick.
[a]Positive values were ≥0.1 kU_A/L (Siemens Immulite 2000/Xpi) and ≥0.35 kU_A/L (EAST).

was the culprit allergy in 59.2%, most frequently triggering urticaria (48.1%). Three patients did not remember the species involved, and 2 also had allergy to chicken (patients 12 and 22) and 1 to crocodile (patient 9). Atopic dermatitis and rhinoconjunctivitis were recorded in 55.5%, asthma in 40.7%, nut allergy in 74%, and shellfish allergy in 44.4%.

The results of SPT to commercial fish extracts and specific IgE (enzyme allergosorbent test [EAST] Siemens Immulite 2000/Xpi) to various fish species are shown in Tables E1 and E2 (Online Repository).

SDS-PAGE IgE-immunoblotting using the sera of all patients and raw food extracts was carried out as described elsewhere [12] and detected a low-molecular-mass IgE-binding protein of ~12 and/or 15 kDa in cod, crocodile, frog, and chicken extracts, revealing a similar molecular mass to those detected by a rabbit anticod parvalbumin serum (Figures E1 and E2, Online Repository). Twenty-five sera recognized a protein of ~12 kDa in the cod extract (Figure 1A, Online Repository). Eighteen and 14 samples recognized both proteins of 12 and 15 kDa in crocodile and frog extracts, and only the 12-kDa band was detected with 2 and 4 sera in crocodile and frog extracts, respectively (Figure E1B and C Online Repository).

A different IgE pattern was observed with chicken meat (Figure E1D, Online Repository). The 12- and 15-kDa bands were detected in 2 cases (patients 12 and 22), and the 15-kDa band was recognized in 14 cases. Other higher-molecular-weight bands were recognized in all meat extracts. Some of these were parvalbumin aggregates, as reported elsewhere [12] (Figure 2, Online Repository).

A ~50-kDa IgE-binding band that was not detected in the cod extract (patient 24) was detected in crocodile, frog, and chicken. An ~18-kDa protein in the cod extract was found in patients 12, 15, 22, and 25; the band was recognized in the crocodile, frog, and chicken extracts in patient 15.

Pools of selected sera with high IgE levels were used to carry out the immunoblotting assays with cooked cod extract (pools 3, 7, 8, 9, 13, 20, and 21), cooked frog extract (pools 3, 10, 13, 14, 17, 18, and 21), cooked crocodile (pools 3, 9, 10, 12, 13, 14, 16, 17, 19, and 21), and cooked chicken (pools 5, 9, 10, 13, 14, 17, and 18). SDS-PAGE immunoblotting with cooked food extracts revealed the same band profiles (between ~16 and 12 kDa) with pools of sera and rabbit anticod parvalbumin serum, as follows: 1 band of ~12 kDa in cod; 2 bands of ~16 and 12 kDa in frog; 3 bands of 16, 13, and 12 kDa in crocodile; and a band of 15 kDa in chicken (Figure E3, Online Repository).

SDS-PAGE immunoblotting-inhibition with the pools of sera and cooked frog, chicken, and crocodile extracts revealed total IgE-binding inhibition with cooked cod extract, thus confirming the presence of cross-reactivity (Figure E4, Online Repository).

We report on a group of fish-allergic patients, some of whom also developed allergy to crocodile and chicken. Allergens were recognized based on variable IgE reactivity.

The immunoblotting assay revealed that most sera detected IgE-reactive protein bands of 12 and/or 15 kDa. The 12-kDa band was the most frequently detected in cod extract, and bands of 12 and/or 15 kDa were the most common in crocodile, frog, and chicken extracts. These may correspond to ß and α parvalbumin, as indicated by the rabbit anticod-parvalbumin results. In addition, bands of 24, 31, and 50 kDa were recognized in cod, crocodile, frog, and chicken extracts in most sera.

Parvalbumin can be detected as a monomer (12 kDa), dimer (24 kDa), trimer (36 kDa), and polypeptide (>40 kDa) and shows remarkable IgE-reactivity [12]. In our study, the same band profile found in these extracts was observed with a cod parvalbumin rabbit antiserum, thus revealing the parvalbumin origin of the bands observed, including parvalbumin monomer and aggregates (Figure E2, Online repository).

Other bands of higher molecular mass (~18 and 50 kDa) were detected in all meat extracts. The ~50-kDa protein may belong to the enolase family, which are allergens in fish and chicken meat [9]. These proteins could all play an important role in the induction of allergic reactions in affected patients.

In summary, crocodile and frog should be considered relevant sources of animal proteins that can act as allergens with clinical relevance owing to their in vitro cross-reactivity and potentially involving other wild exotic foods. Further research is needed to identify and characterize other new allergens.

Funding

The authors declare that no funding was received for the present study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Manuscript received November 16, 2020; accepted for publication May 7, 2021.

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