

Severe anaphylaxis due to crocodile-meat allergy exhibiting wide cross-reactivity with fish allergens



Elisa Haroun-Díaz, MD^a, Natalia Blanca-López, MD, PhD^a,
María Vázquez de la Torre, MD, PhD^a,
Francisco Javier Ruano, MD, PhD^a,
Maria Luisa Somoza Álvarez, MD^a,
Moisés Labrador Horriño, MD, PhD^b,
Borja Bartolomé, PhD^c, Miguel Blanca, MD, PhD^a, and
Gabriela Canto Díez, MD, PhD^a

Clinical Implications

- This is the first report of IgE-mediated allergy to crocodile meat in a child with fish allergy due to parvalbumin in which allergy is clearly demonstrated through *in vivo* and *in vitro* studies.

TO THE EDITOR:

Fish is one of the 8 most important elicitors of food-allergic reactions alongside egg, peanuts, tree nuts, and shellfish.¹ Parvalbumins have been shown to be major fish allergens. It has been suggested that the allergic response to this protein is related to the quantity of the allergen present in the muscle of different fish species.²

Crocodile has been described as a healthy meat source for humans because of its good protein/fat ratio, and it is widely consumed in the southern United States and Australia. In our country (Spain), more and more restaurants are serving this exotic meat.

Members of the Crocodylidae family, known as true crocodiles, are reptiles that live in Africa, Asia, the Americas, and Australia. The term “crocodile” can also refer to members of the order Crocodylia, such as alligators and caimans.

We describe the case of a 9-year-old boy presenting with intense oral itching, perioral erythema, dyspnea, and generalized urticaria within minutes of eating a small portion of crocodile burger for the first time. He required emergency care, receiving adrenaline, diphenhydramine, and oral corticosteroids, and 6 hours after admission was discharged without symptoms.

His medical history included atopic dermatitis and generalized urticaria after fish ingestion at the age of 19 months. Since then, he has avoided fish, but tolerates canned tuna and swordfish as well as chicken and other meats (veal, pork, lamb, turkey, and rabbit).

According to the manufacturer, the burger was prepared with crocodile meat and a powder containing rice, potato, garlic, onion, and spices.

Testing was performed 2 months later. Skin-prick tests to commercial fish extracts (Bial Aristegui) were positive to tuna (5 × 5 mm), cod (9 × 8 mm), anchovy (8 × 8 mm), megrim (13 × 12 mm), sole (9 × 8 mm), hake (9 × 10 mm), anglerfish (9 × 7 mm), salmon (14 × 14 mm), sardine (11 × 11 mm), and

trout (10 × 10 mm), and negative to swordfish. Prick-prick with raw and cooked crocodile was positive: 12 × 12 mm and 9 × 10 mm, respectively. Prick tests with raw crocodile-meat extract (10 × 7 mm) and cooked extract (6 × 6 mm) were positive. See Table E1 in this article's Online Repository at www.jaci-inpractice.org.

Determination of serum-specific IgE (Siemens Immulite 2000/Xpi) showed the following values for fish extracts: cod: 100 kU/L; hake: 15.4 kU/L; tuna: 2.7 kU/L; swordfish: 1.3 kU/L; salmon: 68.0 kU/L; sardine: 5.4 kU/L; sole: 41.7 kU/L; megrim: 8.1 kU/L; trout: 20.6 kU/L. Specific IgE to parvalbumin (r Gad c 1): 24.6 kU/L (ImmunoCAP ISAC). Specific IgE to chicken extract was negative (<0.35 kU/L). Total IgE was 2367 UI/mL. See Table E1 in this article's Online Repository at www.jaci-inpractice.org.

Oral provocation performed with the powder condiment was negative.

Protein extracts from raw and cooked crocodile meat (A), raw hake (B), and raw cod (C) were prepared by homogenization in phosphate-buffered saline, followed by dialysis and lyophilization as described elsewhere.³ Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described by Laemmli.⁴ IgE immunoblotting with the patient's serum revealed a broad IgE-binding band between 10 and 14 kDa in the raw crocodile-meat extract (Figure 1). A similar immunoglobulin profile was detected in this extract with a rabbit antiserum against sardine parvalbumin. Raw cod and hake-extract IgE immunoblots with the patient's serum showed a broad band between 14 and 10 kDa, and others of higher molecular mass. The same immunoglobulin profile was detected in these extracts with a rabbit-antiserum against sardine parvalbumin, revealing the parvalbumin origin of all the bands observed: the broad band is the monomer of parvalbumin, and the others are parvalbumin aggregates (Figure 1). To accurately measure the molecular mass of the crocodile IgE-binding band, SDS-PAGE (16.5% tricine) analysis was performed as described⁵ and an 11-kDa IgE-binding band was detected (Figure 1).

SDS-PAGE immunoblotting inhibition using raw crocodile-meat extract in the solid phase showed complete IgE-binding inhibition when the patient's serum was preincubated with extracts of raw and cooked crocodile meat, cod, and hake (Figure 2).

The 11-kDa IgE-binding band from crocodile was manually excised, digested with trypsin, and analyzed using matrix-assisted laser desorption/ionization time-of-flight spectrometry and liquid chromatography electrospray ionization as by Pastor et al.⁶ Through comparison with a database, the resulting peptides were analyzed using mass spectrometry or tandem mass spectrometry, revealing the identity of some of them with parvalbumin amino acid sequence belonging to different parvalbumin orders: *Xenopus* (*Silurana*), *Pseudopodoces humilis*, *Campylomormyrus compressirostris*, and *Alligator sinensis*.

Parvalbumins are low-molecular-weight Ca²⁺-binding acidic proteins expressed in vertebrates. They have a high resistance to thermal and enzymatic degradation and are found in the cytosol of fast-twitch skeletal muscles and nervous tissue.⁷ Cross-reactions between parvalbumins from fish species are behind polysensitization and allergy to multiple fish species,² and

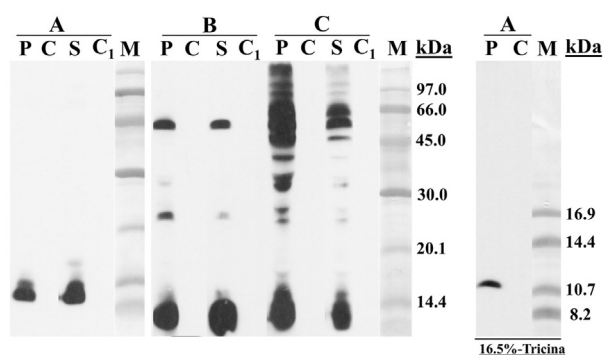


FIGURE 1. SDS-PAGE immunoblotting. **A**, Extract from raw crocodile meat; **B**, extract from raw hake; **C**, extract from raw cod. Lane P: patient's serum, lane C: control serum (pool of sera from nonatopic subjects), lane S: antiparvalbumin rabbit serum. Lane C₁: rabbit serum before parvalbumin immunization, lane M: molecular mass marker. *SDS-PAGE*, Sodium dodecyl sulfate polyacrylamide gel electrophoresis.

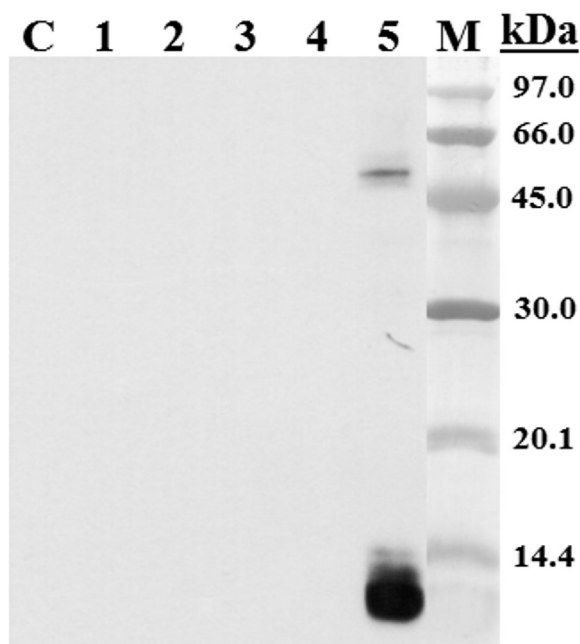


FIGURE 2. SDS-PAGE immunoblotting-inhibition. Raw crocodile meat in solid phase. Lane C: control serum (pool of sera from nonatopic subjects), lanes 1-5: patient's serum previously incubated with extracts from raw crocodile meat (lane 1), from cooked crocodile meat (lane 2), from raw hake (lane 3), from raw cod (lane 4), and from *Helianthus annuus* pollen (lane 5). *SDS-PAGE*, Sodium dodecyl sulfate polyacrylamide gel electrophoresis.

cross-reactivity between parvalbumins of fish with frog⁸ and chicken³ has been reported.

The first case of crocodile-meat allergy due to cross-reactivity between parvalbumin from chicken and crocodile⁹ was recently reported, though no clear information on the allergen responsible for the primary sensitization was provided.

We present a case of a severe anaphylactic reaction after crocodile meat intake. The previous allergic reactions to several fish species observed in this patient, the serum-specific IgE against fish parvalbumin, and the cross-inhibition results suggest fish parvalbumin as the primary sensitization allergen. To our knowledge, this is the first report of crocodile and fish allergy due to cross-reactivity between parvalbumins of both species. Clinicians should be aware of protein cross-reactivity between proteins from fish and reptiles.

Acknowledgments

We would like to thank Musée Gourmet, the patient, and his parents for their collaboration in this case.

^aAllergy Service, Infanta Leonor University Hospital, Madrid, Spain

^bAllergy Unit, Hospital General de la Vall d'Hebron, Barcelona, Spain

^cResearch & Development Department, Roxall, Bilbao, Spain

No funding was received for this work.

Conflicts of interest: The authors declare that they have no relevant conflicts of interest.

Received for publication May 16, 2017; revised July 4, 2017; accepted for publication July 12, 2017.

Available online September 8, 2017.

Corresponding author: Elisa Haroun-Díaz, MD, Allergy Service, Infanta Leonor University Hospital, Av. Gran Vía del Este, 80, 28031 Madrid, Spain. E-mail: elisaharoun@hotmail.com.

2213-2198

© 2017 American Academy of Allergy, Asthma & Immunology

<http://dx.doi.org/10.1016/j.jaip.2017.07.015>

REFERENCES

1. Sicherer SH, Sampson HA. Food allergy. *J Allergy Clin Immunol* 2010;125-(Suppl 2):S116-25.
2. Griesmeier U, Vazquez-Cortes S, Bublin M, Radauer C, Ma Y, Briza P, et al. Expression levels of parvalbumins determine allergenicity of fish species. *Allergy* 2010;65:191-8.
3. Gonzalez-de-Olano D, Bartolome B, Maroto AS, Vivanco F, Pastor-Vargas C. Asthma after chicken consumption due to cross-reactivity between fish and chicken parvalbumin. *J Invest Allergol Clin Immunol* 2012;22:227-8.
4. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680-5.
5. Schagger H, von Jagow G. Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Anal Biochem* 1987;166:368-79.
6. Pastor C, Cuesta-Herranz J, Cases B, Perez-Gordo M, Figueredo E, de las Heras M, et al. Identification of major allergens in watermelon. *Int Arch Allergy Immunol* 2009;149:291-8.
7. Baron G, Demaille J, Dutruge E. The distribution of parvalbumins in muscle and in other tissues. *FEBS Lett* 1975;56:156-60.
8. Hilger C, Thill L, Grigioni F, Lehnert C, Falagiani P, Ferrara A, et al. IgE antibodies of fish allergic patients cross-react with frog parvalbumin. *Allergy* 2004;59:653-60.
9. Ballardini N, Nopp A, Hamsten C, Vetander M, Melen E, Nilsson C, et al. Anaphylactic reactions to novel foods: case report of a child with severe crocodile meat allergy. *Pediatrics* 2017;139(4):e20161404. <https://doi.org/10.1542/peds.2016-1404>.

ONLINE REPOSITORY

TABLE E1. Skin test and specific IgE levels

Allergen	Prick test (mm)	Prick-prick (mm)	Specific IgE (kU/L)
Raw crocodile meat	10 × 7	12 × 12	ND
Cooked crocodile meat	6 × 6	9 × 10	ND
Tuna	5 × 5	ND	2.7
Cod	9 × 8	ND	100
Anchovy	8 × 8	ND	ND
Megrim	13 × 12	ND	8.1
Sole	9 × 8	ND	41.7
Hake	9 × 10	ND	15.4
Anglerfish	9 × 7	ND	ND
Salmon	14 × 14	ND	68.0
Sardine	11 × 11	ND	5.4
Trout	10 × 10	ND	20.6
Swordfish	0	ND	1.3
Parvalbumin (r Gad c 1)	ND	ND	24.6
Chicken	ND	ND	<0.35

ND, Not done.

Reproduced with permission of copyright owner. Further reproduction prohibited without permission.