

An important question is whether EoE-related FTT can be reversed with medical management. Longitudinal data showing the evolution of FTT after diagnosis were available for 14 of the 15 patients. Of these, 8 (62%) resolved their FTT at a median of 18 months after EoE diagnosis (range 3 to 72 months), suggesting medical interventions might have been helpful in these cases. However, because our observations were retrospective and based on a small number of patients, it is difficult to evaluate specific reasons for favorable or unfavorable evolution. All patients underwent many different interventions (including targeted elimination or elemental diet, topical swallowed steroid, short course of oral prednisone, and treatments for other allergic diseases), which makes it difficult to single out the effect of one specific treatment. The fact that FTT did not resolve in 6 patients further underlines the need for new effective therapeutic options in EoE.

Clinical characteristics of our cohort were comparable to previous pediatric series, which suggests it was representative of the pediatric EoE population.⁹ That said, replication in other pediatric EoE cohorts should be performed before results are generalized. One should note that many patients (n = 59) initially referred to our clinic did not meet current clinical and pathologic diagnostic criteria for EoE and thus were excluded from analysis.⁸ This can be explained in part by the long study period, over which EoE criteria have evolved, but it mostly underlines the importance of medical education and of performing a complete investigation before making a definitive diagnosis of EoE in children.

In conclusion, by using various anthropometric criteria to evaluate growth, we found a high rate of FTT in children diagnosed with EoE. Because no single clinical feature can accurately predict it, it is important to maintain a high level of suspicion for FTT in all children with a confirmed or suspected diagnosis of EoE, and inversely, EoE should be included in the differential diagnosis of FTT. Although it resolves in most cases, FTT can persist in some despite medical treatment, highlighting the need for research and new therapies.

Are fish tropomyosins allergens?

Vertebrate tropomyosins are generally not allergenic, with the only exception being tilapia tropomyosin.¹ With a special workup of a patient with fish-induced symptoms, we found that several vertebrate tropomyosins can be allergenic.

Tropomyosin is a highly conserved protein with a wide variety of isoforms in vertebrates and invertebrates. It was the major shrimp allergen in the 1990s but is currently considered an invertebrate pan-allergen. Patients with antitropomyosin IgE against house dust mites or cockroach may have positive skin prick test (SPT) results and oral allergy syndrome after eating shellfish. There are different tropomyosins, doses, and frequencies of exposure (inhalation/ingestion) that are clinically relevant.² Parvalbumins, enolases, aldolases, and fish gelatine are the most important fish allergens.³ However, tropomyosin was not considered a fish allergen until the identification of Ore m 4, the first vertebrate allergenic tropomyosin.¹ The *Anisakis* allergy debate on the necessity of live larvae to elicit allergic symptoms was terminated after it was found that fish with dead larvae are tolerated by patients with previous gastroallergic anisakiasis.⁴

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Supplementary Data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.anai.2015.09.015>.

Brenda Paquet, MD*
Philippe Bégin, MD, MSc*[‡]
Louis Paradis, MD*
Eric Drouin, MD[‡]
Anne Des Roches, MD*[‡]

*Allergy Division

[‡]Service of Gastroenterology, Department of Pediatrics
CHU Sainte-Justine

[‡]CHU Sainte-Justine Research Center
Montreal, Quebec, Canada
a.des.roches@umontreal.ca

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A 19-year-old, white man with a history of house dust mite and shellfish allergy reported ocular redness, epigastralgia, abdominal colic pain, and wheezing 30 minutes after ingestion of several species of fish (Table 1). The patient did not have any digestive disease. Special workup was performed with written consent of the patient and approved by the ethics committee of the Complejo Hospitalario Universitario A Coruña in Spain. At the age of 3 years, he began an atopic march that revealed rhinitis and asthma. When he was 6 years old, SPT and specific IgE data revealed positivity to house dust mites. At this age, he also had oral, pharyngeal, and scalp pruritus and dyspnea when he consumed crustaceans and molluscs. At the age of 13 years, oral pruritus also occurred with octopus and squid. He simultaneously began having symptoms after fish intake. He tolerates canned sardines, anchovies, canned tuna, and smoked or cooked salmon.

A SPT was performed with a battery of allergenic extracts (ALK-Abelló, Madrid, Spain). All positive SPT results had a minimum size of 5 × 6 mm with a histamine control of 5 × 5 mm (Table 1).

His total IgE level was 688 kU/L. Specific IgE determinations by ImmunoCAP (Phadia, Uppsala, Sweden) are listed in Table 1.

Anti-*Anisakis simplex* tropomyosin (Ani s 3) IgE was determined by an antigen-capture enzyme-linked immunosorbent assay (ELISA) using the monoclonal antibody (mAb) TM311 (Sigma, St Louis, MO). Then mAb (1.5 µg per well) was added and

Table 1Serum positivity to tropomyosin, symptoms, and *in vitro* and skin test results against different organisms

Phylum/class	Organism	Skin test result	IgE, kU/L ^a	TRPM	Symptoms
Chordata					
Mammalia	<i>Sus scrofa</i> (pig)	ND	ND	– ^b	No
	<i>Bos taurus</i> (cow)	ND	ND	– ^b	No
	<i>Oryctolagus cuniculus</i> (rabbit)	ND	ND	– ^b	No
Aves	<i>Gallus gallus</i> (chicken)	ND	ND	– ^b	No
Actinopterygii	<i>Lophius piscatorius</i> (monkfish)	+	<0.35	+ ^b	Yes
	<i>Dicentrarchus labrax</i> (European seabass)	ND	ND	– ^b	No
	<i>Oreochromis mossambicus</i> (Mozambique tilapia)	ND	ND	– ^b	ND
	<i>Thunnus alalunga</i> (albacore)	ND	ND	+ ^b	Yes
	<i>Thunnus thynnus</i> (Atlantic bluefin tuna)	–	2.12	– ^b	No
	<i>Xiphias gladius</i> (swordfish)	ND	ND	+ ^b	Yes
	<i>Lepidorhombus whiffiagonis</i> (megrim)	–	2.09	– ^b	Yes
	<i>Merluccius merluccius</i> (European hake)	–	<0.35	+ ^b	Yes
	<i>Gadus morhua</i> (Atlantic cod)	–	ND	– ^b	No
	<i>Salmo salar</i> (Atlantic salmon)	ND	ND	– ^b	No
	<i>Salmo trutta</i> (trout)	ND	ND	– ^b	No
	<i>Engraulis encrasicolus</i> (European anchovy)	ND	ND	– ^b	No
Chondrichthyes	<i>Raja brachyura</i> (blonde ray)	ND	ND	– ^b	Yes
Mollusca					
Bivalvia	<i>Ensis arcuatus</i> (razor clam)	ND	ND	+ ^b	Yes
	<i>Cerastoderma edule</i> (cockle)	ND	ND	+ ^b	Yes
	<i>Ruditapes philippinarum</i> (Japanese littleneck)	ND	12.10	+ ^b	Yes
	<i>Mytilus edulis</i> (blue mussel)	ND	7.15	+ ^b	Yes
Cephalopoda	<i>Loligo vulgaris</i> (squid)	ND	0.57	(+ ^b)	Yes
	<i>Octopus vulgaris</i> (octopus)	+	ND	(+ ^b)	Yes
Arthropoda					
Malacostraca	<i>Palaemon serratus</i> (prawn)	ND	ND	(+ ^b)	Yes
	<i>Litopenaeus vannamei</i> (whiteleg shrimp)	+	ND	+ ^{b,c}	Yes
	<i>Procambarus clarkii</i> (red swamp crayfish)	ND	ND	+ ^b	Yes
	<i>Necora puber</i> (velvet crab)	ND	ND	(+ ^b)	Yes
Arachnida	<i>Dermatophagoides</i> species (house dust mite)	+	45.00	+ ^c	Yes
	<i>Lepidoglyphus destructor</i> (storage mite)	+	28.90	ND	Yes
Insecta	<i>Blattella germanica</i> (German cockroach)	+	ND	+ ^c	Yes
Nematoda					
Chromadorea	<i>Anisakis simplex</i> L3 (herring worm)	+	7.02	+ ^{b,c,d}	ND
	<i>Ascaris suum</i> adults (giant roundworm)	ND	14.20	(+ ^b)	ND
Enoplea	<i>Trichinella spiralis</i> L1 (trichina worm)	ND	ND	(+ ^b)	ND

Abbreviations: L1, first-stage larvae; L3, third-stage larvae; minus sign, negative; ND, not determined; plus sign, positive; TRPM, IgE antitropomyosin.

^aSpecific IgE determination by ImmunoCAP.^bBy immunoblotting.^cBy natural (n) or recombinant (r) tropomyosin ImmunoCAP ISAC (rAni s 3 [106 ISU-E], nPen m 1 [160 ISU-E], rDer p 10 [148 ISU-E], and nBla g 7 [137 ISU-E]). Parentheses indicate putative positivity to IgE antitropomyosin (band not confirmed by TM311).^dBy capture enzyme-linked immunosorbent assay.

incubated and wells incubated with 500 μ g per well of *Anisakis* crude extract. Patient serum (diluted 1:2) was incubated, and a mouse antihuman IgE mAb horseradish peroxidase (HRP)–labeled (clone B3102E8) (SouthernBiotech, Birmingham, Alabama) was added. O-phenylenediamine (Sigma-Aldrich, Darmstadt, Germany) in phosphate-citrate buffer (pH 5.0) with hydrogen peroxide was used as substrate. The reaction was stopped with 3N sulphuric acid, and optical density at 490 nm was read. The patient serum revealed an optical density of 0.279, which is indicative of a positive result.

The absence of positive reaction to recombinant Ani s 1 and a recombinant fragment of Ani s 7 (t-Ani s 7) by indirect ELISA confirmed that the patient was not previously parasitized by *Anisakis* larvae because the recognition of t-Ani s 7 is used as an indicator of previous *Anisakis* infection.⁵

Crude extract preparations were obtained for a wide variety of vertebrate and invertebrate species (Table 1) according to a standard procedure.⁶ After a sodium dodecyl sulfate polyacrylamide gel electrophoresis, crude extracts and the recombinant tropomyosin from the northern shrimp *Pandalus borealis*⁷ (rPan b 1) (25 μ g per lane) were transferred onto nitrocellulose membranes (Hybond ECL; GE Healthcare, Buckinghamshire, United Kingdom). Membranes were incubated with the mAb TM311 followed by a goat antimouse IgG1 HRP conjugate (Molecular Probes Inc, Eugene, Oregon). Membranes were treated with a solution of 3,3'-diaminobenzidine tetrahydrochloride (Sigma-Aldrich) with hydrogen

peroxide in phosphate-buffered saline. For patient IgE determination, the serum was diluted at 1:10, and a murine mAb against human IgE (IgG1 κ , E21A11; INGENASA, Madrid, Spain) followed by a goat antimouse IgG1 HRP conjugate was added. For specific IgG4 and IgA determination, the serum was diluted at 1:25. An anti-human IgG4-HRP mAb (clone HP6025) (SouthernBiotech) or a goat antihuman IgA-HRP (Biosource, Camarillo, California) was used.

IgE antibodies present in the patient serum recognized tropomyosin from hake, monkfish, swordfish, albacore, mussel, razor clam, crayfish, shrimp, Japanese littleneck, and cockle (eFigs 1–7). Specific patterns were confirmed by the mAb TM311, which detected tropomyosin bands. Any fish species tolerated by the patient did not reveal IgE bands in our immunoblotting. On the other hand, those fish that produce the allergic symptoms had IgE bands against tropomyosin (except blonde ray and megrim) (eFig 3A and C, eFig 4C and F, eFig 3E, and eFig 7B). Our patient did not have symptoms with canned tuna or anchovy. The extreme temperature and pressure during canning seem to be responsible for the total absence of protein bands and a decreasing in the allergenicity.

IgE and IgA had a cross-reactive band of approximately 75 kDa in the *Anisakis* crude extract (eFig 1A). This band was also recognized by the antitropomyosin mAb TM311. This fact indicates that *Anisakis* tropomyosin (Ani s 3) is recognized in a dimeric form. These dimeric forms exist in nature in the case of several allergens and might influence allergenicity.^{8,9}

IgG4 antitropomyosin was present in the case of red swamp crayfish, whiteleg shrimp, prawn, velvet crab, blue mussel, Japanese littleneck, octopus, pig, and rabbit crude extracts (only high-molecular-weight homomers). IgA recognition of tropomyosin was observed in the case of Atlantic bluefin tuna, swordfish, albacore, red swamp crayfish, whiteleg shrimp, octopus, blue mussel, and razor clam. The presence of IgE against the described fish tropomyosins was associated with different clinical symptoms after fish ingestion, with a preponderance of digestive and noncutaneous symptoms. Shellfish allergy was not associated with digestive symptoms. We could hypothesize that the absence of protective specific IgA at the intestinal epithelium could be responsible for digestive symptoms. We thus report novelty with respect to the recognition and clinical relevance of several vertebrate fish tropomyosins and the interesting protective IgA isotype.

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Supplementary Data

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Juan González-Fernández, MSc*
Beatriz Veleiro, MD†
Alvaro Daschner, PhD, MD‡
Carmen Cuéllar, PhD*

*Departamento de Parasitología, Facultad de Farmacia
Universidad Complutense

†Servicio de Alergia
Complejo Hospitalario Universitario A Coruña
Coruña, Spain

‡Servicio de Alergia
Instituto de Investigación Sanitaria-Hospital Universitario de La
Princesa
Madrid, Spain
alvarodaschner@gmail.com

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Evaluation of breath alcohol levels after fluticasone nasal spray



Fluticasone propionate nasal spray is widely prescribed for allergic rhinitis. Fluticasone propionate nasal spray, 50 µg, is an aqueous suspension of microfine fluticasone propionate for topical administration to the nasal mucosa by means of a metering, atomizing spray pump. Fluticasone propionate nasal spray also contains microcrystalline cellulose and carboxymethylcellulose sodium, dextrose, 0.02% (wt/wt) benzalkonium chloride, polysorbate 80, and 0.25% (wt/wt) phenylethyl alcohol and has a pH between 5 and 7.

Fluticasone nasal spray is often used at a dosage of 1 to 2 puffs once daily. We tried to determine whether instillation of fluticasone nasal spray, 2 sprays on each side, causes any increase in the breath alcohol levels for a period of 30 minutes. The age group of 16 to 20 years was chosen because the legal limit for drunk driving is 0.02% blood alcohol concentration up to the age of 21 years (0.08% blood alcohol concentration for those older than 21 years).

The breath analyzer model used was Alco-Sensor FST. The Alco-Sensor FST is the model generally used by the law enforcement department to measure alcohol levels in suspected drunk driving cases.

A review of the literature does not reveal any specific study linking fluticasone nasal spray and its effect on blood alcohol concentration in humans.^{1–6} A study by Gomez et al⁷ examined 5 different kinds of nasal sprays, but this study did not involve humans.

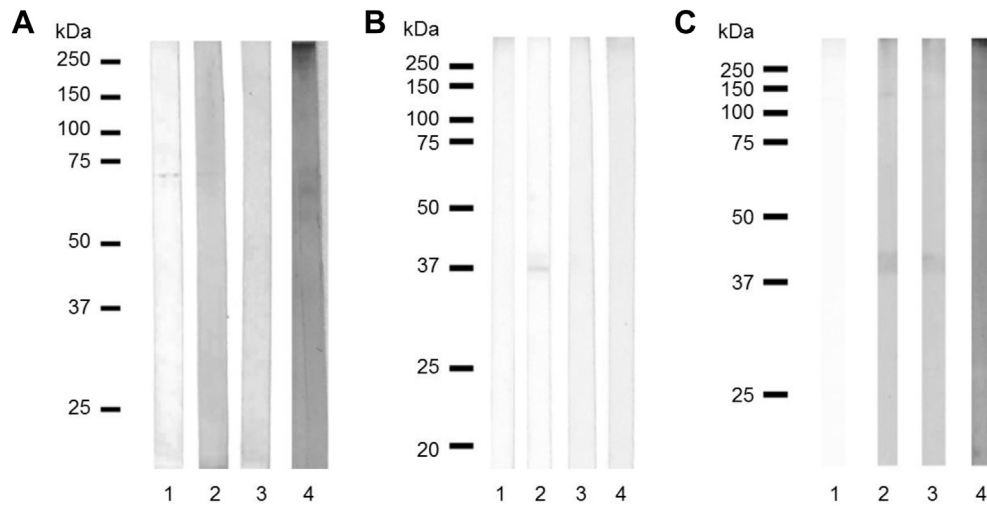
After an extensive search, no data were available addressing the exact metabolism of phenylethyl alcohol after intranasal

administration. The search was limited to review articles and pharmacokinetic studies in humans.

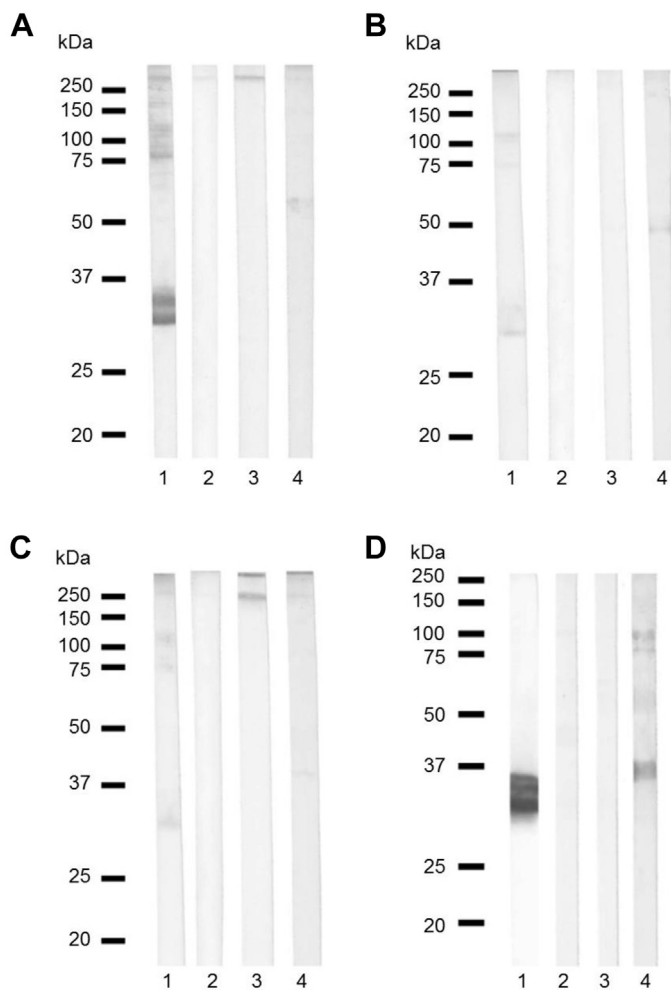
After intranasal administration, a medication can enter systemic circulation through direct local absorption in the mucosa or oral absorption of swallowed material.⁸ In vivo, systemic phenylethyl alcohol is first metabolized to phenylacetaldehyde by multiple NAD⁺ dependent alcohol and aldehyde dehydrogenases. Phenylacetaldehyde is further metabolized to phenylacetic acid by aldehyde dehydrogenase. Phenylacetic acid is further conjugated with glucuronic acid, glutamine, taurine, or glycine and/or eliminated as the free acid. However, the conjugation with glycine, to form phenaceturic acid, has only been evaluated in animal models.^{9,10} Ethyl alcohol is not a metabolite in the degradation pathway.

Healthy adolescents between the ages of 15 and 19 years (9 male and 5 female adolescents) took part in the study. Each adolescent had 2 sprays of fluticasone propionate nasal spray instilled in the nostril on each side. They were screened before the study. The criteria for inclusion into the study were as follows: age of 15 to 19 years; has not had alcohol intake in the past 48 hours in any form, direct or indirect, via any medication; and willing to stay for 60 minutes after the nasal spray is given.

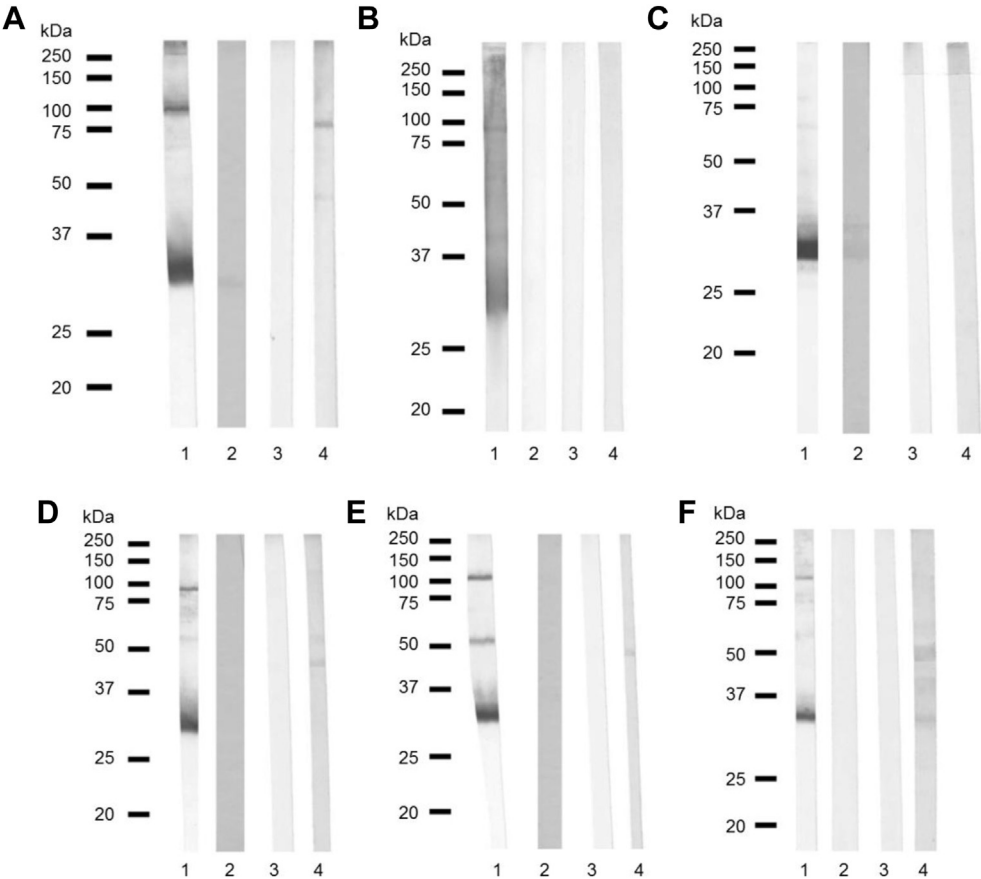
Individuals were excluded if they had demonstrated hypersensitivity to or experienced a serious adverse event in response to fluticasone propionate, had a fever (defined as a temperature >38.0°C) during the past 24 hours, had upper respiratory tract infection symptoms (defined as clear or mucopurulent nasal discharge), had type 1 or type 2 diabetes mellitus, were younger



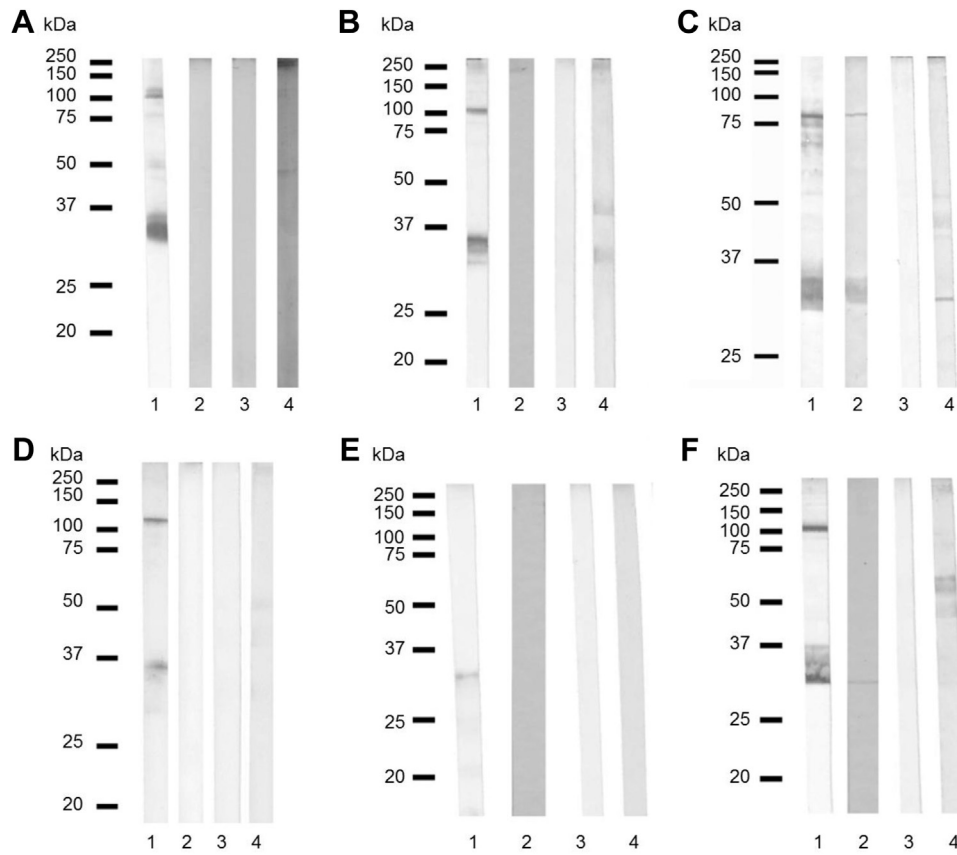
eFigure 1. Immunorecognition patterns of TM311 (lane 1), patient IgE (lane 2), patient IgG4 (lane 3), and patient IgA (lane 4) for *Anisakis simplex* (A), *Trichinella spiralis* (B), and *Ascaris suum* (C).



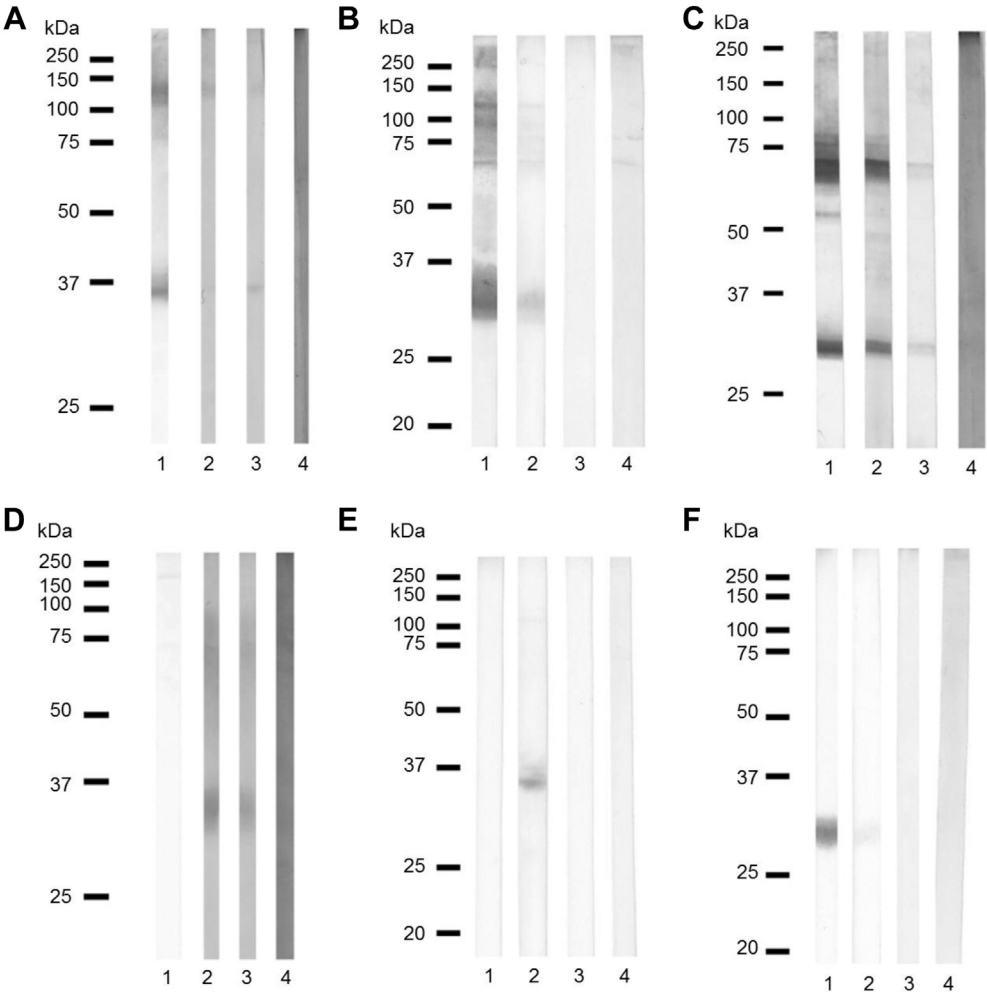
eFigure 2. Immunorecognition patterns of TM311 (lane 1), patient IgE (lane 2), patient IgG4 (lane 3), and patient IgA (lane 4) for pig (A), cow (B), rabbit (C), and chicken (D).



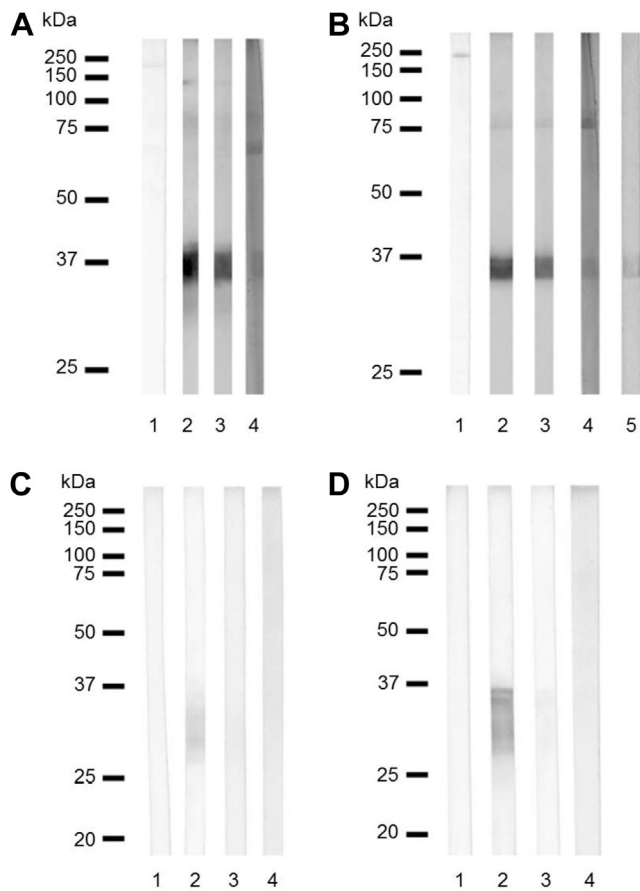
eFigure 3. Immunorecognition patterns of TM311 (lane 1), patient IgE (lane 2), patient IgG4 (lane 3), and patient IgA (lane 4) for hake (A), seabass (B), monkfish (C), cod (D), megrim (E), and tilapia (F).



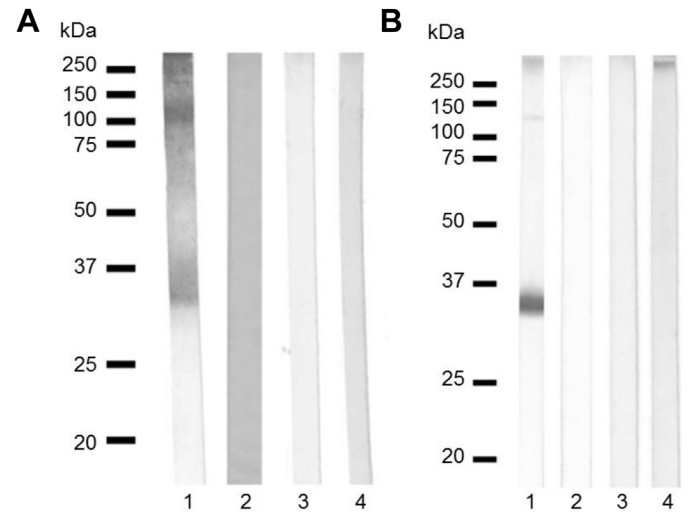
eFigure 4. Immunorecognition patterns of TM311 (lane 1), patient IgE (lane 2), patient IgG4 (lane 3), and patient IgA (lane 4) for tuna (A), trout (B), swordfish (C), salmon (D), anchovy (E), and albacore (F).



eFigure 5. Immunorecognition patterns of TM311 (lane 1), patient IgE (lane 2), patient IgG4 (lane 3), and patient IgA (lane 4) for mussel (A), razor clam (B), Japanese littleneck (C), octopus (D), squid (E), and cockle (F).



eFigure 6. Immunorecognition patterns of TM311 (lane 1), patient IgE (lanes 2 and 5), patient IgG4 (3), and patient IgA (4) for crayfish (A), shrimp (in strip number 5 rPan b 1 was blotted) (B), prawn (C), and crab (D).



eFigure 7. Immunorecognition patterns of TM311 (lane 1), patient IgE (lane 2), patient IgG4 (lane 3), and patient IgA (lane 4) for canned albacore (A) and blonde ray (B).