

infection for our patient remains unknown. However, older age is associated with negative outcomes including hospitalisations, water depletion and even death.⁶ Our patient matches the clinical profile closely given that the patient was dehydrated during the emergency room visit. As such, patients at extreme ages appear more susceptible to the parasite. In fact, longitudinal studies from South America show that children infected with *Cryptosporidium* suffer from growth stunting and lack of weight gain.^{7,8}

Also noteworthy is that the patient's elevated eosinophil count normalised from 3500 to 200 following treatment with nitazoxanole. With a few exceptions, infections caused by protozoans rarely cause eosinophilia. The only two protozoan parasites known to cause peripheral eosinophilia are *Dientamoeba fragilis* and *Isospora belli*. Chronic eosinophilia workup performed in the allergy clinic did not reveal other contributory factors for the patient's abnormal lab finding.

In summary, we have presented a case of a non-immunocompromised adult with a protracted course of diarrhoea attributed to *Cryptosporidium parvum* infection as he responded to anti-parasitic agent therapy with clearing of oocysts from his stools post-treatment. The patient had chronic eosinophilia which resolved with nitazoxanole, and other causes for his chronic eosinophilia had been ruled out. The case highlights the great variability in the clinical presentation of *Cryptosporidium* and the need to consider the diagnosis in immunocompetent individuals suffering from chronic diarrhoea.

Ethical disclosures

Protection of human and animals in research. The authors declare that no experiments were performed on humans or animals for this investigation.

Patient data protection. The authors declare that they have followed the protocols of their work centre on the publication of patient data and that the patient described in this case report has received sufficient information and has given his informed consent in writing to publication about him.

Right to privacy and informed consent. The authors have obtained the informed consent of the patient mentioned in the article. The author for correspondence is in possession of this document.

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Conflicting interests

Dr. Henry Lin and Dr. Joseph Yusin declare no conflicting interests.

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IgE-mediated reaction induced by arugula (*Eruca sativa*) ingestion compared with a spectrum of *Brassicaceae* proteins

To the Editor,

Eruca (E) *sativa* is an edible spontaneous or cultivated plant, native of the Mediterranean area.¹ *E sativa* belongs to

the *Brassicaceae* or *Cruciferae* family, which includes other vegetables such as cabbage, cauliflower, mustard, broccoli, turnip and radish.^{2,3} Arugula is a food of the Mediterranean diet and it is commonly used as a condiment in many dishes (pizza, pasta and salad), consumed raw or cooked.¹

The *Brassicaceae* are able to determine sensitisation and IgE-mediated reactions. Rare cases of adverse reactions after arugula ingestion have been reported, such as contact or systemic manifestations.^{1,4,5}

The aim of this paper is to report an allergic reaction after ingestion of fresh arugula leaves. The study was carried out to demonstrate the IgE-mediated mechanism of the reaction referred and to evaluate the co-sensitisation to other edible *Brassicaceae*, realizing their protein profile.

A 32-year-old housewife reported urticaria to the trunk a few minutes after ingestion of pizza with raw arugula. History revealed allergic respiratory symptoms and no previous adverse reactions to foods. The reaction resolved with antihistamine treatment. The ingestion of other types of pizza did not generate problems. Skin prick test (SPTs) with commercial aeroallergen and food extracts (Stallergenes, Milan, Italy) were performed. Histamine and saline solution were used as positive and negative controls, respectively. The SPTs were positive to different aeroallergens (grass pollen, mugweed, *Parietaria judaica*, olive tree pollen, cypress, amaranth, cat dander and *Alternaria alternata*) and foods (peanut, potato and shrimp). The patient did not report food allergies; she ate foods with positive SPTs without reaction.

In addition, prick by prick test with raw arugula was performed with positive result. The prick by prick tests with raw arugula were also performed in ten healthy controls and ten atopsics, with negative results.

The patient refused to undergo oral challenge test with raw arugula. The proteins were extracted from raw and cooked arugula leaves,⁶ with a concentration approximately of 6.314 mg/ml and 0.885 mg/ml, respectively. The gel electrophoresis protein profile, after staining with 0.1% Coomassie brilliant blue, showed bands with molecular weight approximately of 17.6, 67.5, 83.7, 127.8 kDa for raw arugula extract and 59.7 and 73.9 kDa for boiled arugula extract (Fig. 1).

To explain the prick by prick test positive with raw arugula, the proteins were transferred onto nitrocellulose membrane and incubated with patient's serum overnight. Specific IgE binding was detected by peroxidase-conjugated anti-human IgE antibodies from goat and luminol. The immunoblotting (IB) analysis did not show IgE reactivity to proteins in the cooked extract, while bands reactive were presented to the protein of about 60–67 kDa of the raw arugula extract (Fig. 1). To exclude the possibility of an unspecific reaction, IB analysis performed with control serum was negative (Fig. 1).

To evaluate the co-sensitisation between other foods belonging to the *Brassicaceae* family, SPTs with commercial extracts of mustard (Stallergenes, Milan, Italy), cabbage and cauliflower (Lofarma, Milan, Italy), and prick by prick test with cabbage, cauliflower and turnip, both raw and boiled, and raw radish (because in our region this vegetable is usually eaten in its native state) were performed. Determination of specific IgE (RAST) for the same food was also performed. SPTs with commercial extracts and RAST were negative, while the prick by prick tests resulted positive for raw *Brassicaceae*. The patient did not report reactions after ingestion of cooked turnips and cauliflower, and she did not remember having eaten radish and/or cabbage. The protein extracts, obtained from other *Brassicaceae*,⁶ were used to perform gel electrophoresis (Fig. 2).

In the literature, cutaneous and/or respiratory symptoms induced by arugula have rarely been reported. In all cases,

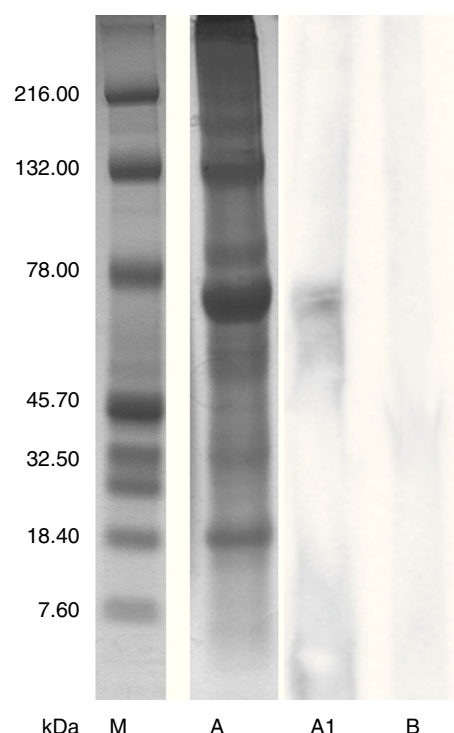


Figure 1 *Eruca sativa* protein identification. Molecular markers (lane M). SDS-PAGE with extracts of raw *E. sativa* (lane A) and immunoblotting with serum from the *E. sativa* sensitive patient (lane A1). Negative control serum (lane B).

the diagnosis was confirmed by prick by prick test with the native food.^{1,4,5}

In a few cases, IB has been used to better investigate the adverse reactions induced by the arugula. In one case, IB was performed to demonstrate a possible cross-reactivity between arugula and pollens¹ and in another case,

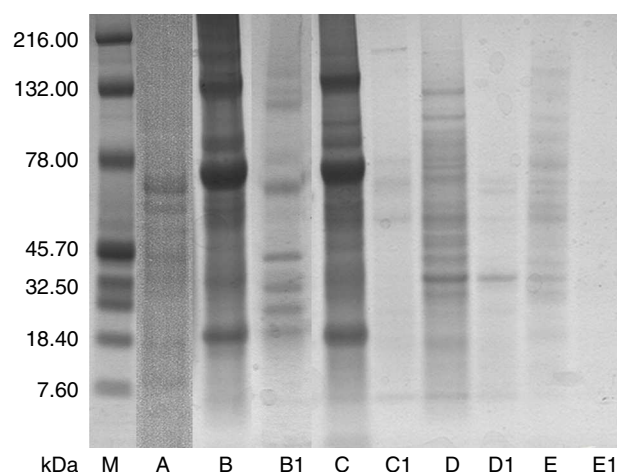


Figure 2 Spectrum of *Brassicaceae* proteins. Molecular markers (lane M). Extracts of radish (lane A), raw and boiled arugula (lane B and B1), raw and boiled turnips (lane C and C1), raw and boiled cauliflower (lane D and D1) and raw and boiled cabbage (lane E and E1).

instead, to assess IgE reactivity in patient's serum. In this last case, however, no IgE reactive was detected despite the patient having had a positive SPTs with arugula extract prepared in the laboratory. To explain this contradiction, Foti et al. suggested that this difference may be related to the manner in which the extract for the IB has been prepared.⁵

The prick by prick tests with other cooked and raw *Brassicaceae* showed positivity only for raw foods, assuming the presence of thermolabile protein, as suggested by some authors.² The analysis of gel protein profile with raw and cooked extracts showed a different protein profile with reduction or loss of protein in the boiled extracts (Fig. 2). Furthermore, with the exception of radish and arugula, in our country these vegetables are consumed cooked. In fact, the patient tolerated the ingestion of cooked cauliflower and turnips.

It is possible to note a similarity of the spectrum of the protein of raw arugula and raw turnip, as shown in Fig. 2. Then, it is possible to hypothesize cross-reactivity between these vegetables because the prick by prick tests were also positive.

In conclusion, the study shows the IgE-mediated mechanism of the reaction lamented by patient, because prick by prick test with raw arugula was positive and the IB showed IgE reactivity in the patient's serum against the protein of molecular weight approximately 60–67 kDa. In addition, the study of co-sensitisation to other foods belonging to the *Brassicaceae* family has confirmed the presence of proteins inactivated by heating by: prick by prick test positive for only raw vegetables, the different protein profile between the raw and cooked vegetables extracts and the tolerance of the cooked *Brassicaceae*, as already previously assumed.²

Allergic reactions can be induced to any foods because they contain proteins and, then, can be studied by IB.⁷ This laboratory technique is therefore a useful diagnostic tool when the commercial extract or substrate RAST is not available.^{8,9}

Further studies should be performed to better define the allergenic proteins of these foods and possible cross-reactivity between vegetables of the *Brassicaceae* family.

Ethical disclosures

Protection of human subjects and animals in research. The authors declare that no experiments were performed on humans or animals for this investigation.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflict of interest

All authors have read and approved the final version of the manuscript. All authors meet the criteria specified in the request for authorship. All authors certify that they have collectively and/or personally written at least 90 percent of manuscript. The authors declare that the manuscript is original and has not been published previously in print/electronic format or in another language and that the manuscript is not under consideration by another publication or electronic media. The authors declare that they have no financial support.

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