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Case report

Food-induced anaphylaxis to *Tenebrio molitor* and allergens implicated

Anaphylaxie alimentaire à Tenebrio molitor et allergènes en cause

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Abstract

The first reported French case of severe food anaphylaxis induced by *Tenebrio molitor* (mealworm) concerns a male patient whose history consisted solely of allergy to house dust mites. He was not sensitised to crustaceans. Proteomic analysis of his serum led us to investigate *T. molitor* proteins to which he was sensitised, and, in particular, pupal cuticle protein G1A, which has never previously been notified as an allergenic protein of *T. molitor*. The patient also appeared to be sensitised to larval cuticle proteins A1A and A2B, to hexamerin, and to tropomyosin epitopes uninvolved in mite or crustacean cross-reactivity. He also appeared to be sensitised to α-amylase in *T. molitor*, the three-dimensional structures and sequences of which resemble those of the house mite *Dermatophagoides pteronyssinus*, and also to tubulin, a potential pan-allergen. We therefore draw attention to the risk for mite-allergic patients of eating *T. molitor* larvae, and we stress the urgent need for strict food labelling regulations for edible insects.

Keywords: Food anaphylaxis; Food allergy; *Tenebrio molitor*; Yellow mealworm; Insect foods; Entomophagy; *Tenebrio molitor* allergens; House dust mites

Résumé

Le premier cas français d'anaphylaxie alimentaire sévère à la larve de ténébrion est un patient aux antécédents exclusifs d'allergie aux acariens de la poussière de maison, non sensibilisé aux crustacés, et qui a présenté une réaction anaphylactique après ingestion d'une seule larve de ténébrion (ver de farine). L'exploration protéomique de son sérum nous a permis d'étudier les protéines de ténébrion auxquelles il est sensibilisé, et en particulier, semble-t-il, une protéine qui n'avait encore jamais été signalée comme allergène du ténébrion: la protéine G1A de la cuticule de la pupe. Le patient semble également sensibilisé aux protéines A1A et A2B de la cuticule de la larve, à l'hexamérine, à la tropomyosine du ténébrion dont l'épitope reconnu chez lui n'est toutefois pas un épitope commun avec les crustacés ni avec les acariens. Il semble également sensibilisé à l'alpha-amylase du ténébrion, qui a une forte homologie de structure et de séquence avec celle de l'acarien *Dermatophagoides pteronyssinus*, et à la tubuline qui serait un pan allergène potentiel. Nous attirons donc l'attention sur le risque que représente la consommation de larves de *T. molitor* pour les patients allergiques aux acariens, et la nécessité urgente d'une réglementation rigoureuse de l'étiquetage alimentaire concernant les insectes comestibles.

Mots clés : Anaphylaxie alimentaire ; Allergie alimentaire ; Ténébrion ; *Tenebrio molitor* ; Ver de farine ; Insectes comestibles ; Entomophagie ; Allergènes de *Tenebrio molitor* ; Acariens de la poussière de maison

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1. Introduction

Tenebrio molitor, or mealworm, is an arthropod belonging to the insect class and is of the Coleoptera order.

Insect consumption, an important source of simple, low-cost protein, is common in many countries [1,2]. The legislation authorizing such consumption differs from country to country [3–5]. In France, the use of insects or insect proteins is currently allowed under certain conditions for animal feed, in particular, aquaculture. Its authorisation for human consumption has been governed since 1 January 2018 at the European level by Regulation (EU) N° 2015/2283, whose application in France is under the control of the DGCCRF (General Directorate for Competition Policy, Consumer Affairs and Fraud Control) [3].

A number of cases of anaphylaxis following ingestion or inhalation of this arthropod have been described, with *T. molitor* being also used as bait by anglers [6,7]. In 2014, Van der Brempt and Moneret Vautrin warned of the possible allergic risk of *T. molitor* if used as food by humans [8]. A report by the ANSES (the French Agency for Food, Environmental and Occupational Health & Safety) also called for vigilance [2].

Cases described hitherto mostly share a point in common with tropomyosin sensitisation, with risk of allergy to *T. molitor* having been demonstrated in shrimp-allergic patients [9,10].

The first French case of severe anaphylaxis to *T. molitor* larva allowed us to identify the allergenic proteins responsible in this patient.

2. Clinical case study

2.1. Disease history

The patient was a 31-year-old man who, at a business seminar, was offered cooked larvae, probably fried, as an appetizer. He ingested one of these and drank 25 cl of beer. He ate nothing else.

Fifteen minutes later, on sitting down for dinner, he presented urticaria, which began on his neck before spreading rapidly, with angioedema of the face and extremities to the point of having to remove his watch. After 25 minutes, laryngeal dyspnoea and nausea occurred.

He was rushed to the nearest hospital by emergency services, where he underwent adrenaline infusion. His symptoms subsided within an hour but he was placed on sick leave for the ensuing 24 h.

2.2. Previous history

The patient had no previous history of food allergy. Since his childhood, he had presented rhinitis only in certain old houses, as well as mild asthma, but with very few attacks. He had never previously undergone any allergological tests.

He was taking no ongoing treatment for asthma and his current spirometry results were completely normal.

He was also presenting hiatal hernia, for which he was taking esomeprazole 40 mg/d.

2.3. Suspected food

The patient himself learned from the hotel chef that what he had eaten were in fact mealworm larvae (*T. molitor*) supplied by the company Jiminy's®.

2.4. Allergy tests

The prick-tests were highly positive for the dust mites *Dermatophagoides pteronyssinus* (papule diameter: 10 mm) and *Dermatophagoides farinae* (10 mm), positive for *T. molitor* flour (5 mm), doubtful for grasshopper flour (3 mm), and negative for cricket flour (these food-grade flours were supplied by JC). The histamine control was 7 mm.

The shrimp prick-test was negative.

Specific IgE (ImmunoCAP™ reagents, ThermoFisher Scientific) was positive for mealworm (1.11 kU/L), dust mites *D. pteronyssinus* (27.6 kU/l) and *D. farinae* (27 kU/l), and for allergenic components rDer p 1 (9.82 kU/l), rDer p 2 (10.60 kU/l), and rDer p 23 (1.85 kU/l).

In the case of tropomyosins, rPen was 1 < 0.10 kU/l and rDer p 10 was < 0.10 kU/l.

2.5. Diagnosis

The diagnosis made was thus of grade 2 food anaphylaxis to mealworm (*T. molitor)* following ingestion of a single cooked larva in a patient sensitised to dust mites but not sensitised to crustaceans, nor to shrimp tropomyosin.

The patient usually consumes a lot of crustaceans, which he has eaten without any problems since the anaphylactic reaction to *T. molitor*.

It was noted that the reaction was possibly aggravated by two co-factors: ingestion of alcohol and of esomeprazole (proton pump inhibitor).

This case was notified to the Allergy Vigilance Network® (Réseau d'Allergo-Vigilance®).

3. Additional proteomic investigations

In order to identify the *Tenebrion* proteins to which the patient was sensitised, his serum was sent to the HELMo Centre for Grouped Research Institutes (CRIG/HELMo) in Liège, which carried out various analyses.

3.1. SDS-PAGE and Western blot

First, gel electrophoresis (SDS-PAGE or sodium dodecyl sulphate polyacrylamide gel electrophoresis) was run with a *T. Molitor* extract and enabled the proteins to be separated from the extract by molecular-weight (1D SDS-PAGE), and then by isoelectric point and molecular-weight (2D SDS-PAGE) (Fig. 1).

1D Western blot (1DWB) and 2D Western blot (2D WB) analyses were then performed.

The Western blot technique involves transferring to a membrane (impregnated with serum from the patient containing his

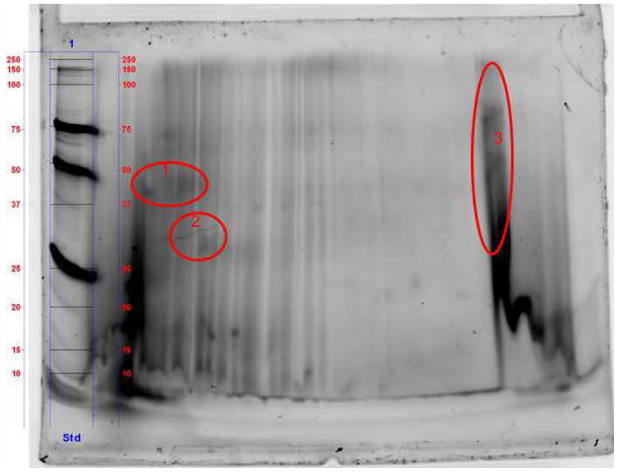

Fig. 1. 2D SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis) with highlighting of protein spots identified in mass spectrometry. The protein spots in circles 1 and 2 were identified as tropomyosin while those in circle 3 were identified as hexamerin 2.

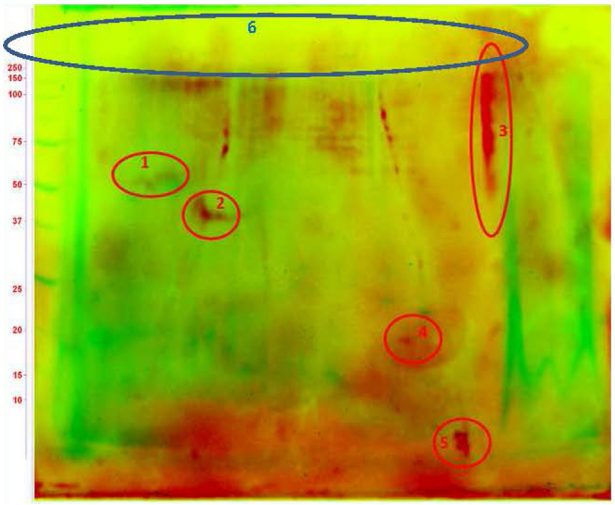

Fig. 2. 2D Western blot. Overlay of 2D SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis) gel and PVDF (polyvinylidene difluoride) membrane. Highlighting of reaction zones, indicating proteins to which the patient is sensitised. Zones 1–3 are those shown in Fig. 1. Zone 4 may correspond to pupal cuticle protein G1A, while zone 5 may correspond to larva cuticle proteins A1A and/or A2B. Zone 6 may indicate sensitivity to chitin.

or her specific antibodies, called "primary antibodies") gel proteins that will react with this serum. The reaction zones are then highlighted using secondary antibodies directed against these primary antibodies.

1D WB enabled two reactive zones to be demonstrated: a first high molecular-weight zone of around 50-100 kDa, and a low molecular-weight zone of around 10-18 kDa.

The proteins described in the literature having a molecular-weight of around 15 kDa correspond to *Tenebrio* cuticular proteins. Indeed, there are already reports in the literature indicating that *Tenebrio* cuticular protein A1A has a molecular-weight of 17.7 kDa and that *Tenebrio* cuticular protein A2B protein has a molecular-weight of 12.3 kDa.

The proteins described in the literature having a molecular-weight of between 50 and 100 kDa may correspond to α-amylase (51.7 kDa), the α-1 chain of tubulin (50.6 kDa), hexamerin 2 (84.5 kDa), and/or haemocyanin (90.6 kDa) [11].

2DWB, which is more precise, is used with two-dimensional electrophoresis, and allows more accurate identification of the molecular allergens responsible for an allergy in a given patient. At this stage of the analysis, assumptions about the identity of the proteins in question are based on searching for analogies of the experimentally determined physicochemical characteristics with regard to databases available in the literature (Uniprot and Allergome).

2DWB demonstrated six reactive zones for the *Tenebrio* extract (Fig. 2).

Zone 1 had an isoelectric point (IEP) of ± 3 and a molecular-weight (MW) of around 50 kDa, which could correspond to tubulin α-1 chain. In the same zone, a protein with an IEP of ± 5 and MW of 51.7 kDa could correspond to α-amylase.

Zone 2 had an IEP of ± 4 and a MW of around 35 kDa, which could correspond to tropomyosin.

Zone 3 had an IEP of ± 9 and a MW of around 100 kDa (probably several isoforms at this level), which could correspond to haemocyanin and/or hexamerin 2.

Zone 4 had an IEP of ± 7 and a MW of around 20 kDa, which could correspond to pupal cuticle protein G1A.

Zone 5 had an IEP of ± 8 and a MW of around 15 kDa, which could correspond to larval cuticle protein A1A or to larval cuticle protein A2B.

A sixth zone, clearly visible in the 2DWB, appears to correspond to chitin, a non-protein allergen which is a non-soluble polysaccharide.

3.2. Mass spectrometry analysis

Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is a sensitive and specific technique. Liquid chromatography separates the various components present in a mixture using a column, enabling analysis of the specific compounds present. The mass spectrometer uses three quadrupoles (Q1-q2-Q3), i.e. two quadrupole analysers coupled in series and separated by a collision cell. The first quadrupole, Q1, allows the isolation of a "parent" ion on the basis of its mass-to-load ratio (m/z). The second quadrupole, q2, serves as a collision cell whose role is to fragment "parent" compounds, allowing greater sensitivity and specificity based on the m/z ratio of a compound and its fragments. Quadrupole Q3 is used to analyse "product" ions based on their m/z ratio. LC-MS/MS produces an ionic chromatogram, which is a graphical representation of the analysis of a sample, whose signal intensity is detected throughout the time axis [12].

Using this technique, we were able to confirm the identity of proteins in two areas reacting with the patient's serum and to determine that he was sensitised to hexamerin 2 and to *Tenebrio* tropomyosin. It should be noted that the patient was probably also sensitised to cuticle proteins based on the protein spots seen in 2DWB, although these molecules could not be accurately identified by mass spectrometry.

4. Discussion

This is thus a patient with no previous history of food allergy, sensitised simply to house dust mites *D. pteronyssinus* and *D. farinae*, and who developed grade 2 anaphylaxis following his first ingestion of a single larva of *T. molitor*.

In attempting to elucidate the causative mechanism, we formulated the following two hypotheses:

The first hypothesis is that of primary anaphylaxis to one or more allergens specific to *T. molitor*. In this hypothesis, the possibility of prior sensitisation by contact or inhalation during angling activities was ruled out by questioning. It would thus seem that the patient was unwittingly sensitised by means of food contamination following ingestion of flour containing particles of *T. molitor*.

The second hypothesis is that of anaphylactic reaction through cross-allergy to house dust mites via allergens common to *T. molitor* and house dust mites.

Analysis of the laboratory results shows that this patient differs from the cases previously described in that he is not sensitised to shrimp tropomyosin pen A1, although cross-allergy between *T. molitor* and crustaceans is frequently reported in the literature [9,10,13,14]. Nor is he sensitised to dust mite tropomyosin Der p 10. However, his IgE recognize a 35-kDa protein that is indeed a tropomyosin having several isoforms, but which does not cause cross-allergy with Pen A1 or Der p 10.

His IgE also recognize *T. molitor* hexamerin. Hexamerins are circulating proteins found in the haemolymph of insect larvae [15].

The study by A. Barre et al. in 2016 [16] referred to arginine kinase, α-amylase, glutathione-S transferase and tropomyosin as cross allergens frequently revealed by Western blotting.

No IgE against arginine kinase or glutathione-S transferase was detected in our patient. However, IgE directed against α-amylase was indeed present, as revealed by an area corresponding to a protein of 51.7 kDa. α-amylase was identified as an allergen in mealworm by Debaugnies et al. in 2016 [17]. Using a 3D model, Barre et al. demonstrated structural homology between *T. molitor* α-amylase and that of the dust mite *D. pteronyssinus*, Der p 4 [16,18].

The patient also appears to have IgE directed against the α-1 chain of *T. molitor* tubulin according to 2DWB, although this could not be confirmed by mass spectrometry. Tubulins are structural proteins found in invertebrate cytoskeleton, and the dust mite *D. farinae* also contains an α- tubulin named Der f 33 [18,19].

In 2DWB (Fig. 2), the patient appears to be sensitised to larva cuticle proteins A1A and A2B, already identified as *T. molitor* allergens [14]. In 2017, Broekmann et al. described food-induced anaphylaxis in two *T. molitor* breeders with high respiratory exposure over a long period (7 to 9 years) and who ingested large quantities (50 g per day). Both patients were sensitised to larval cuticle proteins A1A, A2B and A3A, and the authors concluded that these proteins could be the dominant allergens in primary mealworm allergy [20].

2DWB also shows apparent sensitisation of our patient to pupal cuticle protein G1A. This new finding is of interest since we found no reference to this phenomenon in the literature. However, the identity of allergens A1A, A2B and G1A could not be accurately confirmed using MS.

Finally, our patient may have been sensitised to chitin, a non-protein allergen, but no formal identification could be made.

5. Conclusion

This first French case of severe food-induced anaphylaxis to *T. molitor* larva in a patient allergic to house dust mites but not sensitised to crustaceans, following ingestion for the first time in his life of a cooked, whole mealworm larva, enabled us to examine the allergenic proteins of *T. molitor.*

Among these proteins, α-amylase and tubulin at the very least are common pan-allergens, having strong structure and sequence homologies and potentially cross reactive with house dust mites.

Larval cuticle proteins A1A and A2B appear to be primary mealworm allergens, but cross-allergenicity studies with house dust mites should be considered.

In this patient, cuticle protein G1A appears to be a possible new mealworm allergen.

In view of these results, while the possibility of pre-sensitisation by unwitting ingestion of mealworm as a masked allergen cannot be ruled out, cross-allergenicity between mealworm and dust mites doubtless played an important role in the genesis of this severe anaphylaxis.

With the increasingly frequent consumption of mealworm, which is sold either over-the-counter by certain retailers turning a blind eye to the legislation or via the Internet, comes the risk of an increase in cases of anaphylaxis, particularly in patients allergic to dust mites. It is therefore urgent that strict regulations be introduced concerning the food labelling of insects and insect proteins.

Disclosure of interest

The authors declare that they have no competing interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.reval.2019.06.001.

References

[1] Van der Brempt X, Beaudouin E, Lavaud F. Manger des insectes, est-ce dangereux pour l'allergique? Rev Fr Allergol 2016;56:186–8.
[2] https://www.anses.fr/fr/system/files/BIORISK2014sa0153.pdf.
[3] https://eur-lex.europa.eu/legal-content/FR/TXT/PDF/?uri=CELEX:32015R2283&rid=1.
[4] https://info.agriculture.gouv.fr/gedei/site/bo-agri/instruction-2017-860 Direction générale de l'Alimentation. Utilisation des protéines et autres produits dérivés d'insectes dans l'alimentation humaine, animale, et pour des usages techniques (2017).
[5] AFSCA (Agence fédérale pour la sécurité de la chaîne alimentaire), Circulaire relative à l'élevage et à la commercialisation d'insectes et de denrées à base d'insectes pour la consommation humaine. [Circular on the rearing and marketing of insects and insect-based foods for human consumption.

Belgian Federal Agency for the Safety of the Food Chain (AFSCA)]. 2014;p.1-9.
[6] Freye HB, Esch RE, Litwin CM, Sorkin L. Anaphylaxis to the ingestion and inhalation of *Tenebrio molitor* (mealworm) and *Zophobas morio* (superworm). Allergy Asthma Proc 1996;17:215–9.
[7] Bernstein DI, Gallagher JS, Bernstein IL. Mealworm asthma: clinical and immunologic studies. J Allergy Clin Immunol 1983;72:475–80.
[8] Van der Brempt X, Moneret-Vautrin DA. Le risque allergique de *Tenebrio molitor* pour la consommation humaine. Rev Fr Allergol 2014;54:34–6.
[9] Broekman HCHP, Knulst AC, de Jong G, Gaspari M, den Hartog Jager CF, Houben GF. Verhoeckx KCMet. Is mealworm or shrimp allergy indicative for food allergy to insects? Mol Nutr Food Res 2017;61(9).
[10] Broekman H, Knulst A, Den Hartog Jager S, et al. Shrimp allergic patients are at risk when eating mealworm. Clin Transl Allergy 2015;5(Suppl 3):77.
[11] Grebe SK, Singh RJ. LC-MS/MS in the Clinical Laboratory–Where to From Here? Clin Biochem Rev 2011;32:5–31.
[12] Rusconi F. Manuel de spectrométrie de masse à l'usage des biochimistes. Lavoisier 2011.
[13] Verhoeckx KCM, Van Broekhoven S, den Hartog-Jager CF, Gaspari M, de Jong GAH, Wickers HJ. House dust mite (Der p 10) and crustacean allergic patients may react to food containing Yellow mealworm proteins. Food Chem Toxicol 2014;65:364–73.
[14] de Gier S, Verhoeckx K. Insect (food) allergy and allergens. Mol Immunol 2018;100:82–106.
[15] Just N, Lièvre K, Lallemand K, Leduc V. Implication de l'hexamérine dans un cas d'allergie aux larves de mouche. Rev Fr Allergol 2012;52:258.
[16] Barre A, Velazquez E, Delplanque A, Caze-Subra S, Bienvenu F, Bienvenu J, Benoist H, Rougé P. Les allergènes croisant des insectes comestibles. Rev Fr Allergol 2016;56:522–32.
[17] Debaugnies F, Francis F, Delporte C, Doyen V, Ledent C, Mairesse M, et al. Identification de l'α-amylase comme allergène du ver de farine chez des patients professionnellement exposés. Rev Fr Allergol 2016;56:281.
[18] Barre A, Simplicien M, Cassan G, Benoist H, Rougé P. Food allergen families common to different arthropods (mites, insects, crustaceans), mollusks and nematods: Cross-reactivity and potential cross-allergenicity. Rev Fr Allergol 2018;58:581–93.
[19] http://www.allergome.org/script/dettaglio.php?id_molecule=11692.
[20] Broekman HCHP, Knulst AC, den Hartog Jager CF, van Bilsen JHM, Raymakers FML, Kruizinga AG, et al. Primary respiratory and food allergy to mealworm. J Allergy Clin Immunol 2017;140:600–3.