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BRIEF COMMUNICATION

Evaluation of the theoretical risk of cross-reactivity among recently identified food allergens for dogs

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Abstract

Background: There is increasing evidence of cross-reactivity between allergens of close or distant species. The A-RISC (Allergens'–Relative Identity, Similarity and Cross-reactivity) index helps evaluate the risk of theoretical cross-reactivity between proteins of the same family among different species.

Objectives: To report the A-RISC indices for several food allergens of dogs between multiple food sources.

Materials and Methods: We selected several recently characterised food allergens for dogs from fish and chicken (ACTA1, ALDOA, CKM, ENO3, GAPDH, PKM and TPI1), fish (TPM1/2), beef/lamb (PGM1) and corn/potato (WAXY). When quality sequence data were available, A-RISC indices were calculated between multiple animal and plant species that can be used as food sources. For the TPM subunits, A-RISC indices also were calculated with the environmental allergens Bla g 4 and Der f 10, and the *Toxocara canis* nematode.

Results: The A-RISC indices suggest a substantial theoretical risk of cross-reactivity between species for all allergens considered. For TPM, this risk also extends to the environmental and nematode allergens.

Conclusions and clinical relevance: There is a high theoretical risk of cross-reactivity between allergens of different species used as food sources. The clinical relevance of these elevated A-RISC indices should be studied further.

INTRODUCTION

At this time, the diagnosis of food allergies (FA) in pets relies on the performance of an open, two-phase, restriction–provocation trial.[1] During the restriction phase, the pet normally is given a home-made or commercial diet formulated with 'novel', 'limited', 'restricted' or 'single protein' ingredients, or protein digests of variable degree of hydrolysis (i.e. hydrolysates). Because of the higher costs of the latter, clinicians often opt for the lower-priced 'novel protein' diets. Still, such a selection presumes that the novel diet does not share any cross-reactive allergens with the originally eaten food.

Unfortunately, each publication characterising new food allergens in dogs raises increasing concerns that an extensive cross-reactivity might exist among food allergens. Indeed, the main food sources causing allergies in dogs are meats of mammalian, poultry or piscine origin,[2] and, consequently, newly characterised immunoglobulin (Ig)E-targeted allergens are muscle or blood proteins. While the first discovery of meat allergens in dogs suggested the immunological (IgE) cross-reactivity between two allergens of evolutionary-close species (e.g. beef, lamb and cow's milk),[3] a recent study established the presence of multiple IgE-cross-reactive allergens between chicken and two evolutionarily

Abbreviations: A-RISC, Allergens' Relative Identity, Similarity and Cross-reactivity; FA, food allergies.

distant fish species.[4] In 2022, we reported the identification of eight chicken proteins targeted by serum IgE from dogs with clinical signs of chicken allergy.[5] For one of these allergens (chicken serum albumin, ALB, Gal d 5), we used a novel index the A-RISC (Allergens'–Relative Identity, Similarity and Cross-reactivity)[6] to help determine the theoretical risk of cross-reactivity of this chicken allergen with homologous proteins from other poultry, mammalian, fish and reptile (alligator) species. As the A-RISC indices often were higher than 50% (0.50), the chance of cross-reactivity among albumins thus was predicted to vary from medium to very high.[5] While a clinical cross-reactivity was not proven—or even investigated—in the dogs from that study, these elevated A-RISC indices raised the question that a cross-reactivity might exist for some food-allergic dogs. Indeed, if a dog were IgE-sensitised to chicken's Gal d 5, there is a risk that its clinical signs might not decrease following the feeding of, for example, alligator- (A-RISC = 0.68) or rabbit- (A-RISC = 0.52) based diets.

This paper expands the A-RISC index calculations to other previously published meat and plant allergens. The values obtained suggest a very high theoretical risk of cross-reactivity among food allergens, even from evolutionarily distant species.

MATERIALS AND METHODS

From previous reports of IgE-targeted allergens in sensitised dogs,[3–5] we selected the following meat allergens: alpha-actin (ACTA1), aldolase A (ALDOA), creatine kinase M (CKM), (beta) enolase 3 (ENO3), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), L-lactate dehydrogenase (LDHA), phosphoglucomutase 1 (PGM1), protein-kinase M (PKM) and triosephosphate isomerase 1 (TPI1). We excluded Bos d 7 (bovine IgG), the first beef/milk allergen identified in dogs,[3] due to its multiple-chain structure and the presence of IgG subclasses, making comparisons between species difficult. We also omitted Bos d 6 (bovine serum albumin), which we discussed in detail in our previous paper.[5] Whenever good quality amino acid sequences were available, A-RISC indices were calculated, as done previously,[6] between the following species: chicken (*Gallus domesticus*), duck (*Anas* spp.), turkey (*Meleagris gallopavo)*, ostrich (*Struthio camelus*), cow (*Bos domesticus*), sheep (*Ovis aries*), horse (*Equus caballus*), pig (*Sus domesticus*), rabbit (*Oryctolagus cuniculus*), alligator (*Alligator Mississippiensis*), salmon (*Salmo salar*) and cod (*Gadus morhua*).

As an illustration that cross-reactivity phenomena are not limited to meat allergens, we added the IgE-targeted granule-bound starch synthase 1 (WAXY), which is found in starches from several hydrolysate-containing commercial diets.[7] For this allergen, we determined the A-RISC indices between the following plant foods: wheat (*Triticum aestivum*), barley (*Fagopyrum esculentum*), rice (*Oryza sativa*), corn/maize (*Zea mays)*, potato (*Solanum tuberosum*) and pea (*Pisum sativum*).

Finally, to demonstrate that cross-reactivity also exists between food allergens and proteins from the same family in evolutionarily distant insect, arachnid and nematode species, we calculated the A-RISC indices for the two subunits of the tropomyosin allergen (TPM1 and TPM2) between homologous proteins of the animal species described above, to which we added those of the *Dermatophagoides farinae* house dust mite (Der f 10), the German cockroach (*Blatella germanica*, Bla g 7) and the ubiquitous *Toxocara canis* roundworm.

RESULTS

In Figure 1a, we show the A-RISC indices for LDHA, which we selected as a representative meat allergen. The theoretical risk of cross-reactivity between the examined species was high to very high, because indices ranged from 0.77 to 0.98. As can be expected, the indices were highest between evolutionarily close species (i.e. between selected mammals, poultry or fish) and lower between these different groups. This segregation between groups became more obvious when plotting the different sequences of LDHA against that of chicken (Figure 1b).

Nearly identical results were obtained when calculating A-RISC indices for the other meat allergens ACTA1, ALDOA, CKM, ENO3, GAPDH, PGM1, PKM and TPI1 (Figure S1).

For our representative plant food allergen, the GBSS1 (WAXY), the theoretical risk of allergen cross-reactivity also was substantial between examined species (Figure 1c); the A-RISC indices were highest among grains and also elevated between grains, potato and pea. When compared to the wheat's WAXY sequence, that of barley was the closest, followed by corn and rice, and then potato and pea (Figure 1d).

Finally, we calculated A-RISC indices for the two chains of TPM, a well-known panallergen for humans and a recently found fish allergen in dogs.[8] As can be seen in Figure S1, the high-value indices suggested a substantial risk of cross-reactivity between mammal, poultry and fish TPMs. This risk also was important between the TPMs of these species and two inhalant allergens, Bla g 7 and Der f 10, the latter being a minor mite allergen for dogs.[8] Finally, A-RISC indices indicated a possible cross-reactivity, albeit lower, between all of these TPMs and that of *Toxocara canis*.

DISCUSSION

We report herein the theoretical risk of cross-reactivity between several allergens from multiple animal and plant species using the newly developed A-RISC index.[6]

As meats represent the main sources that trigger food allergies in dogs and cats,[2] we first focussed on the muscle proteins recently characterised as allergens.[3–5,8] The A-RISC indices among species were high to very high, thus suggesting a substantial likelihood of cross-reactivity. The additional A-RISC calculations for the WAXY and TPM allergens further highlighted that

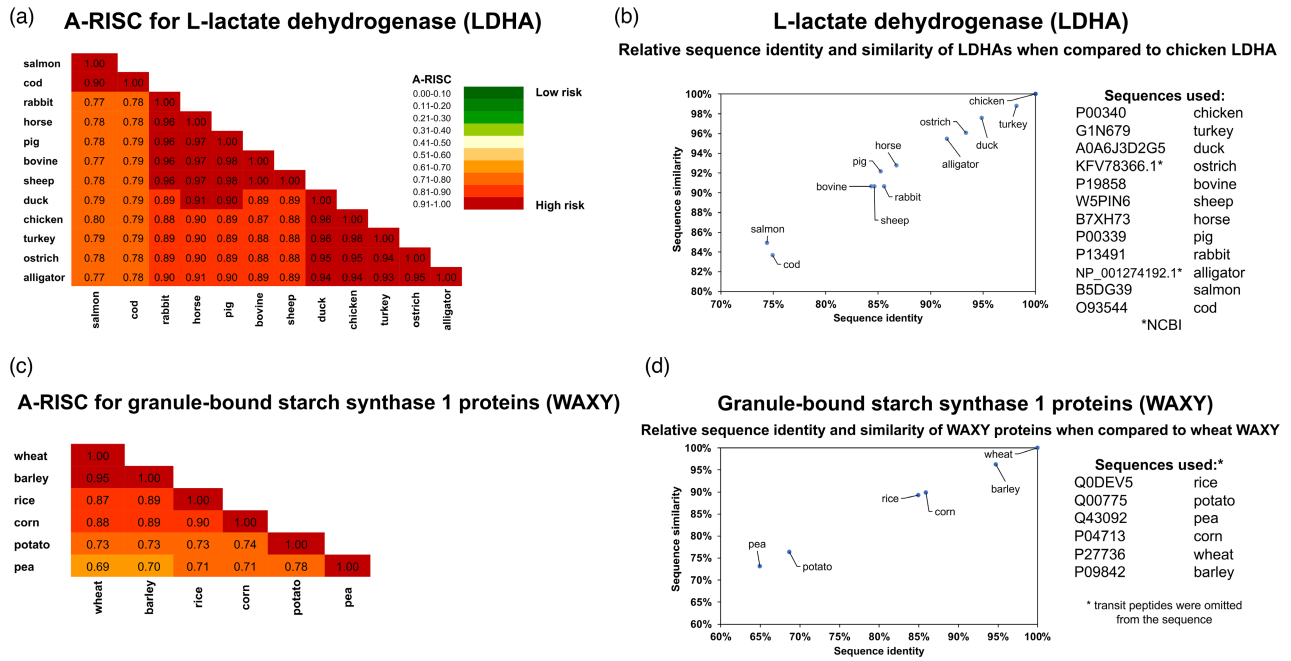

FIGURE 1 A-RISC (Allergens'–Relative Identity, Similarity and Cross-reactivity) indices for l-lactate dehydrogenase (LDHA) and granule-bound starch synthase 1 (WAXY). (a) A-RISC indices for LDHA between 12 animal species can be used as different food sources. (b) When compared to chicken, the closest LDHA sequences are those of birds and alligators, then mammals and then fish species. (c) A-RISC indices for WAXY between six plants used as food sources; (d) when compared to wheat, the WAXY sequence of barley is closest; then, we find those of rice and corn and then potato and pea.

such cross-reactivity is not limited to meat allergens and also might occur between meat, nematode and environmental allergens.

It is unknown if these theoretical predictions will translate into a clinical cross-reactivity when dogs eat foods from different origins, yet there is cause for concern.

In some cases, the pertinence of the A-RISC already is supported by immunological and clinical data. For example, the A-RISC index between the ENO3 of chicken (Gal d 9) and those of cod (Gad m 2) and salmon (Sal s 2) is 0.84, which suggests a high theoretical risk of cross-reactivity between these two types of meats. The recent demonstration of cross-reactivity between chicken and fish at the immunological (humans and dogs)[4,9] and clinical (humans)[9] levels validates the relevance of the notional risk determined by the A-RISC indices.

Although theoretical, the potential cross-reactivities between the allergens of all these foods should prompt the reexamination of the current practice of performing restrictive diets for diagnosing food allergies with 'novel, limited, restricted' food sources. Indeed, on the one hand, if clinical signs improve with these diets and recur after provocation with the original food, clinicians can confidently diagnose food allergies. However, on the other hand, if clinical signs do not diminish with the newly introduced diet, the clinician will be left to wonder if the diagnosis of food allergy can be excluded, or alternatively, if the lack of improvement is due to the feeding of cross-reactive food, environmental or nematode allergens to which it is sensitised.

Consequently, the performance of food trials with 'novel' diets is inherently fraught with a sensitivity for the diagnosis of FA that probably is lower than that currently expected; it is thus evident that the real prevalence of FA in animals likely is higher than that reported.[10]

In conclusion, we report herein the elevated theoretical risk of allergenic cross-reactivity between allergens of multiple animal and species used as food sources for pets. Further studies should help determine whether these risks correlate with clinical allergy.

AUTHOR CONTRIBUTIONS

Thierry Olivry: Conceptualization; Writing – original draft; Supervision. **Andrea O'Malley:** Investigation; Writing – review & editing. **Maksimilian Chrusz:** Investigation; Methodology; Validation; Visualization; Writing – review & editing.

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CONFLICT OF INTEREST

No conflicts of interest have been declared.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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Résumé

Contexte: Il y a de plus en plus de preuves de réactivité croisée entre des allergènes d'espèces proches ou éloignées. L'indice A-RISC (Allergens'–Relative Identity, Similarity and Cross-reactivity) permet d'évaluer le risque théorique de réactivité croisée entre protéines d'une même famille parmi différentes espèces.

Objectifs: Rapporter les indices A-RISC pour plusieurs allergènes alimentaires de chiens entre plusieurs sources alimentaires.

Matériels et méthodes: Nous avons sélectionné plusieurs allergènes alimentaires récemment caractérisés pour les chiens parmi les poissons et poulet (ACTA1, ALDOA, CKM, ENO3, GAPDH, PKM, TPI1), poisson (TPM1/2), bœuf/agneau (PGM1) et maïs/ pomme de terre (WAXY). Lorsque des données de séquence de qualité étaient disponibles, les indices A-RISC ont été calculés entre plusieurs espèces animales et végétales pouvant être utilisées comme sources de nourriture. Pour les sous-unités TPM, les indices A-RISC ont également été calculés avec les allergènes environnementaux Bla g 4 et Der f 10, et le nématode *Toxocara canis*.

Résultats: Les indices A-RISC suggèrent un risque théorique important de réactivité croisée entre espèces pour tous les allergènes considérés. Pour le TPM, ce risque s'étend également aux allergènes environnementaux et aux nématodes.

Conclusions et pertinence clinique: Il existe un risque théorique élevé de réactivité croisée entre allergènes d'espèces différentes utilisées comme sources alimentaires. La pertinence clinique de ces indices A-RISC élevés doit être étudiée plus avant.

Resumen

Introducción: Cada vez hay más evidencia de reactividad cruzada entre alérgenos de especies cercanas o distantes. El índice A-RISC (Identidad, similitud y reactividad cruzada relativa de alérgenos) ayuda a evaluar el riesgo de reactividad cruzada teórica entre proteínas de la misma familia entre diferentes especies.

Objetivos: publicar los índices A-RISC para varios alérgenos alimentarios de perros entre múltiples fuentes de alimentación.

Materiales y Métodos: seleccionamos varios alérgenos alimentarios para perros recientemente caracterizados de pescado y pollo (ACTA1, ALDOA, CKM, ENO3, GAPDH, PKM, TPI1), pescado (TPM1/2), ternera/cordero (PGM1) y maíz/ patata (WAXY). Cuando los datos de secuencias de calidad estaban disponibles, se calcularon los índices A-RISC entre múltiples especies de animales y plantas que pueden usarse como fuentes de alimento. Para las subunidades TPM también se calcularon índices A-RISC con los alérgenos ambientales Bla g 4 y Der f 10, y el nematodo *Toxocara canis*.

Resultados: Los índices A-RISC sugieren un riesgo teórico sustancial de reactividad cruzada entre especies para todos los alérgenos considerados. Para TPM, este riesgo también se extiende a los alérgenos ambientales y de nematodos.
Conclusiones y relevancia clínica: Existe un alto riesgo teórico de reactividad cruzada entre alérgenos de diferentes especies utilizadas como fuente de alimento. La relevancia clínica de estos índices A-RISC elevados debe estudiarse más a fondo.

Zusammenfassung

Hintergrund: Es gibt zunehmende Evidenz, dass eine Kreuzreaktivität zwischen Allergenen von nahen oder weiter voneinander entfernten Spezies besteht. Der A-RISC (Allergens´-Relative Identity, Similarity and Cross-Reactivity) Index hilft bei der Evaluierung des Risikos theoretisch auftretender Kreuzreaktivität zwischen Proteinen derselben Familie bei unterschiedlichen Spezies.
Ziele: Ein Bericht über die A-RISC Indices für mehrere Futterallergene bei Hunden in verschiedenen Futtermitteln.
Materialien und Methoden: Wir wählten mehrere unlängst beschriebene Futterallergene für Hunde von Fisch und Huhn (ACTA1, ALDOA, CKM, ENO3, GAPDH, PKM, TPI1), Fisch (TPM1/2), Rind/Lamm (PGM1) und Mais/Kartoffel (WAXY). Wenn Daten der Qualitätssequenz vorlagen, wurden A-RISC Indices zwischen verschiedenen tierischen und pflanzlichen Spezies kalkuliert, die als Futterquelle Verwendung finden können. Für die TPM Untergruppen wurden A-RISC Indices ebenso kalkuliert wir die Umweltallergene Bla g 4 und Der f 10, sowie die Nematode *Toxocara canis*.
Ergebnisse: Die A-RISC Indices weisen auf ein deutliches theoretisches Risiko einer Kreuzreaktivität zwischen den Spezies für alle untersuchten Allergene hin. Für TPM erstreckt sich das Risiko auch auf Umwelt- und Nematodenallergene.
Schlussfolgerungen und klinische Bedeutung: Es besteht ein hohes theoretisches Risiko einer Kreuzreaktivität zwischen den Allergenen verschiedener Spezies, die als Futterquellen Verwendung finden. Die klinische Relevanz dieser erhöhten A-RISC Indices sollte weiter untersucht werden.

要約

背景: 近縁または遠縁種アレルゲン間の交差反応性を示す証拠が増えてきている。A-RISC(Allergens'-Relative Identity, Similarity and Cross-reactivity)指標は、異種間における同一ファミリーのタンパク質間の理論的交差反応性のリスクを評価するのに役立つ。
目的: 本研究の目的は、犬の食物アレルゲンについて、複数の食物源間の A-RISC 指標を報告することであった。
材料と方法: 我々は、魚と鶏(ACTA1, ALDOA, CKM, ENO3, GAPDH, PKM, TPI1)、魚(TPM1/2)、牛肉/ラム(PGM1)、トウモロコシ/ジャガイモ(WAXY)から最近特徴づけられたいくつかの犬の食物アレルゲンを選択した。質の高い配列データが得られた場合には、食料源として利用可能な複数の動植物種間でA-RISC指標を算出した。TPMサブユニットについては、環境アレルゲンのBla g 4とDer f 10、および*Toxocara canis*線虫とのA-RISC指標も算出した。
結果: A-RISC指標は、検討したすべてのアレルゲンについて、種間の交差反応性の理論的リスクを示唆している。TPMについては、このリスクは環境アレルゲンおよび線虫アレルゲンにも及んでいる。
結論と臨床的関連性: 食物源として使用される異種アレルゲン間には、高い理論的交差反応性リスクが存在する。これらのA-RISC指数の上昇と臨床的関連性については、さらなる研究が必要である。

摘要

背景: 来越多的证据表明近缘或远缘物种的过敏原之间存在交叉反应性。A-RISC(过敏原-相对同一性、相似性和交叉反应性)指数有助于评价不同种属间同一家族蛋白之间理论交叉反应性的风险。
目的: 告多种食物来源之间犬的几种食物过敏原的 A-RISC 指数。
材料和方法: 们从鱼和鸡 (ACTA1、ALDOA、CKM、ENO3、GAPDH、PKM、TPI1)、鱼 (TPM1/2)、牛肉/羔羊 (PGM1) 和玉米/马铃薯 (WAXY) 中选择了几种相近表征的犬用食物过敏原。当质量序列数据可用时 , 计算可用作食物来源的多种动植物之间的 A-RISC 指数。对于 TPM 亚基 , 还用环境过敏原 Bla g 4 和 Der f 10 以及犬弓首蛔虫线虫计算了 A-RISC 指数。
结果: RISC指数表明 , 对于考虑的所有过敏原 , 种属间交叉反应性的理论风险较大。对于TPM , 这种风险也扩展到环境和线虫过敏原。
结论和临床相关性: 作食物来源的不同种属的过敏原之间的交叉反应性理论风险较高。应进一步研究这些 A-RISC 指数升高的临床相关性。

Resumo

Contexto: Há evidências crescentes de reatividade cruzada entre alérgenos de espécies próximas ou distantes. O índice A-RISC (*Allergens'-Relative Identity, Similarity and Cross-reactivity* – índice de identidade relativa, similaridade e reação cruzada de alérgenos) ajuda a avaliar o risco de reatividade cruzada teórica entre proteínas da mesma família entre espécies diferentes.

Objetivos: Relatar os índices A-RISC para vários alérgenos alimentares de cães de múltiplas fontes alimentares.

Materiais e Métodos: Selecionamos vários alérgenos alimentares para cães recentemente caracterizados oriundos de peixes e frango (ACTA1, ALDOA, CKM, ENO3, GAPDH, PKM, TPI1), peixes (TPM1/2), carne bovina/cordeiro (PGM1) e milho/batata (WAXY). Quando havia dados de sequenciamento de boa qualidade disponíveis, os índices A-RISC foram calculados entre várias espécies animais e vegetais utilizadas como fontes de alimento. Para as subunidades TPM, também foram calculados índices A-RISC com os alérgenos ambientais Bla g 4 e Der f 10, e o nematoide *Toxocara canis*.

Resultados: Os índices A-RISC sugerem um risco teórico substancial de reatividade cruzada entre espécies para todos os alérgenos considerados. Para o TPM, esse risco também se estende aos alérgenos ambientais e nematóides.

Conclusões e relevância clínica: Existe um alto risco teórico de reatividade cruzada entre alérgenos de diferentes espécies utilizadas como fontes alimentares. A relevância clínica destes elevados índices A-RISC deve ser mais estudada.