

Results of food challenge in dogs with cutaneous adverse food reactions

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Background – Elimination diet trials and food challenge tests remain the gold standard for diagnosing cutaneous adverse food reaction (CAFR) in dogs. However, food challenge tests are difficult to perform owing to a lack of definite information on expected clinical signs and the time frame within which they develop after challenge.

Objectives – To report the results of the food challenge test carried out in client-owned dogs under observation.

Methods and materials – A retrospective evaluation of the medical records of dogs presenting with pruritus was performed. Skin infections and/or parasites were treated before inclusion in the study. Dogs that underwent an elimination diet with clinical improvement and relapse with food challenge tests were included in this study. A pruritus Visual Analog scale score was recorded as an index of pruritus before and at the end of the elimination diet trial before challenge. The onset of time to relapse of clinical signs and the body sites affected after food challenge testing were recorded by the owners.

Results – We examined the medical records of 310 dogs with pruritus. Of these, 46 dogs with a diagnosis of CAFR were enrolled in this study. Significant clinical improvement was observed after the elimination diet trial ($P < 0.0001$). All dogs exhibited pruritus, and none exhibited anaphylaxis after food challenge testing. Among these dogs, 60.9% developed pruritus within 12 h of food challenge. Notably, 23.9% of the dogs developed pruritus within 3–6 h. The limbs exhibited the highest frequency of pruritus (56.5%), followed by the face (26.1%).

Conclusions and clinical relevance – This study is the first to report details of the time to onset of clinical signs and body site affected after food challenge testing in dogs. These results will assist veterinarians and dog owners in the decision-making process regarding food challenge testing.

Introduction

Cutaneous adverse food reaction (CAFR) is a common skin disease which affects many dogs.¹ For effective long-term management, identification of the inciting food (s) is important. Several *in vivo* and *in vitro* tests, such as the intradermal test, prick test, serum immunoglobulin (Ig)E test and lymphocyte proliferation test, have been trialled to identify the causative allergen. However, scientific studies so far have failed to show these tests to be reliable in practice.² At the present time, elimination diet trials followed by food challenge tests remain the gold standard for diagnosing CAFR.²

In humans, food challenge testing can result in varied clinical signs ranging from mild cutaneous signs to systemic and potentially life-threatening reactions.³ Therefore, the risk of food challenge testing has been investigated in detail.⁴ Probably because a food challenge is rarely associated with severe adverse effects in dogs, such studies have not been performed. Additionally, a

specific problem in veterinary medicine is that many owners are reluctant to perform food challenges once they perceive improvement in their pet's quality of life on the elimination diet.

The aim of this study is to characterise the owner-observed adverse reactions to food challenge in a population of client-owned dogs.

Methods and materials

Ethics

This study observed the Japanese national guidelines for the humane treatment of animals. All owners provided written informed consent for the study.

Study population

Client-owned dogs with chronic pruritus and a diagnosis of CAFR were included in this prospective cohort study from January 2019 to September 2019. The diagnosis was made after the exclusion of infectious and parasitic diseases, and after complete remission of clinical signs on an elimination diet trial followed by worsening of clinical signs with the food challenge test.

Concurrent disease was identified and ruled out according to published methodology.⁵ All dogs were subject to treatments relevant to their clinical signs and diagnostic findings. This included miticidal trials and flea control (fluralaner orally two times in six months (Bravecto, MSD Animal Health K. K.; Tokyo, Japan)). Concurrent secondary superficial bacterial pyoderma and *Malassezia* dermatitis

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were diagnosed by skin surface cytological evaluation (tape preparations and/or impression smears) and treated initially with systemic antibiotics or antifungal therapy as indicated. Cefalexin (25 mg/kg twice daily for 28 days) or fosfomycin (20 mg/kg twice daily for 28 days) was administered based on the results of susceptibility testing or, in the case where *Malassezia* was present on cytological evaluation, itraconazole 5 mg/kg once daily for 28 days was prescribed.

Elimination diet trial

An elimination diet trial was performed in each dog for four weeks using a single protein source selected on the basis of the dog's previous nutritional history. LABOLINE Pure Protein Salmon (Zenoaq; Fukushima, Japan) or Allergic Therapeutic Food Horse (UEHARA HC Co., Inc.; Gunma, Japan). Other treatments were not allowed during the trial period. A pruritus Visual Analog Scale (pVAS) score was recorded as an index of pruritus before and after four weeks on the elimination diet trial before challenge.⁶

Food challenge test

The food challenge testing was performed when the clinical signs had improved on the elimination diet trial ($pVAS \leq 2$) and was started on the first day when the owners could monitor the dogs at home for three consecutive days. The dogs were challenged with food that they had eaten before the elimination diet was performed. Each dog consumed the original food during the challenge until the clinical signs recurred. The owner was asked to record the time at which the first clinical signs were noted and the body site affected after the food challenge was initiated. If clinical signs recurred, the owner was asked to discontinue the food challenge test and to administer prednisolone at home [1 mg/kg twice daily for five days (Predonine, Shionogi & Co., Ltd; Osaka, Japan)]. If the clinical signs did not improve with prednisolone, the dog was treated at the hospital.

Statistical methods

Statistical analysis was performed using a paired Student's *t*-test. Statistical processing was performed using PRISM 5.0 (GraphPad Software Inc.; San Diego, CA, USA). A *P*-value of <0.05 was considered significant.

Results

We examined 310 pruritic dogs, and 46 dogs with CAFR were enrolled in the study (see Table S1 in Supporting information). In addition to pruritus, the dogs with CAFR showed clinical signs such as hair loss, erythema and papules. Twenty-three dogs were fed elimination diets containing salmon and the remaining 23 were fed horse meat. Among the dogs enrolled, 65.2% (30 dogs) were male. The mean body weight was 8.67 kg and the mean age at presentation was 5.79 years. The most common breed in the study was the toy poodle (15 dogs), followed by the French bulldog (five dogs) and miniature dachshund (three dogs). Two dogs each were of the breeds bichon frise, golden retriever and pug. Two dogs were mixed breed, and all other breeds occurred only once.

Significant improvement in pruritus was observed after the elimination diet trial using food composed of novel proteins ($P < 0.0001$; Figure 1). The mean pVAS score was 6.68 [standard deviation (SD) 2.11] at the time of the first diagnosis. A pVAS score of 6.0 denotes a state of constant itching when the dog is not excited. After the elimination diet trial, the mean pVAS score decreased to 0.89 (SD 0.57), which is within the normal range.

After the food challenge test, all 46 dogs exhibited pruritus. No other signs were observed in this study. The median time to relapse was 12 h (range 1.5 h to 10 days).

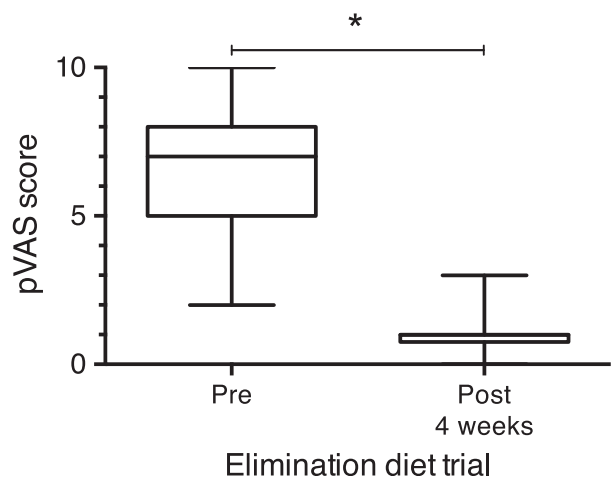


Figure 1. Mean pruritus Visual Analog Scale (pVAS) score before and after the elimination diet trial.

The degree of pruritus at the site of the lesion for all dogs was estimated using the pVAS scoring system. The asterisk denotes significant differences between the pre-treatment and post-treatment scores ($P < 0.0001$).

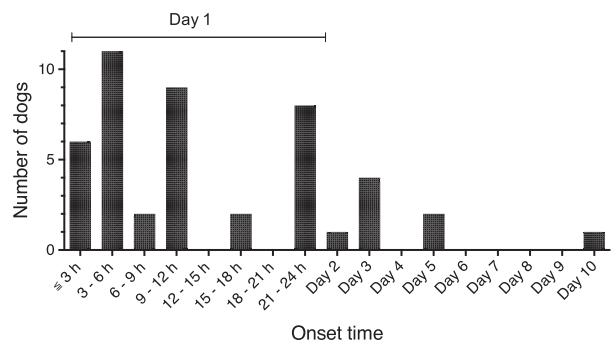


Figure 2. Time of onset of clinical signs after food challenge testing. The dogs were categorized every 3 h on Day 1.

Among the 46 dogs, 60.9% showed pruritus within 12 h (Figure 2). Notably, many dogs developed pruritus within 3-6 h (23.9%). Among the 46 dogs, one, four and two had onset on day (D)2, D3 and D5, respectively. One dog developed itching on D10.

Pruritus occurred at particular body sites (Figure 3). The limbs were the most common site (56.5%), followed by the face (26.1%). Moreover, 19.6%, 17.4%, 17.4% and 10.9% of the dogs developed pruritus on the lips, eyelid, ear pinnae and neck, respectively. The frequency of pruritus on the truncal sites was $<10\%$ (chest 4.3%; abdomen 8.7%; dorsolumbar area 4.3%; femur 2.2%; axillae 2.2%; groin 4.3%; margin of the anus 6.5%; and tail 2.2%). None of the dogs developed anaphylaxis.

When clinical signs recurred, all dogs were fed the elimination diet and treated with prednisolone (1 mg/kg for five days). After five days, all dogs were in remission and prednisolone was discontinued. None of the dogs needed additional treatment.

Discussion

Our study followed a cohort of 46 dogs that were diagnosed with CAFR based on a significant improvement in

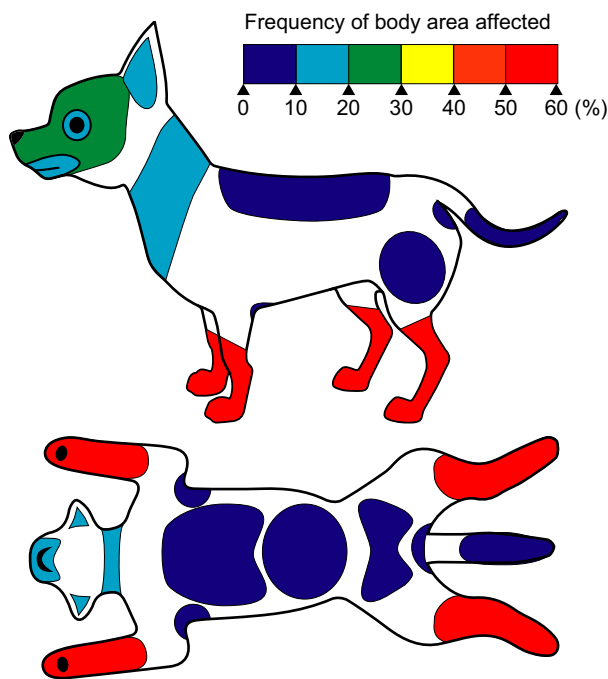


Figure 3. Illustration of the body sites affected by pruritus on dogs after food challenge testing.

Each colour corresponds to the frequency of that body area being affected. Blue, <10%. light blue, >10% and <20%; green, >20% and <30%; yellow, >30% and <40%; orange, >40% and <50%; and red, >50% and <60%.

clinical signs whilst on the elimination diet and subsequent relapse on challenge with foods fed previously. Furthermore, as a consequence of close follow-up with the owners, we were able to document the time to relapse and body sites affected. After challenge initial clinical signs of pruritus developed in every case, and quick intervention with prednisolone and discontinuation of the offending food resulted in resolution of the pruritus and no further clinical signs developing. Although pruritus is reportedly the predominant clinical sign noted in CAFR recurrent pyoderma, otitis externa and pyotraumatic dermatitis also have been reported.⁷ We did not see these signs after challenge, presumably owing to swift pharmacological intervention. Regarding severe symptoms, such as anaphylaxis, a previous study showed that dogs with experimental sensitization to the allergen did not show any severe cutaneous or respiratory symptoms after the oral food challenge, which is similar to our results.⁸ In humans anaphylaxis is more common and close supervision is required during food challenge testing.³ Our study suggests that such measures are not necessary when a food challenge test is performed in dogs.

Most dogs diagnosed with a CAFR are pruritic, most often in a generalized pattern, with the face, ears, feet and abdomen frequently affected.^{7,9} It should be noted, however, that these studies reported on dogs with chronic CAFR. The present study indicated that the limbs and the area around the face are especially common pruritic sites immediately after a food challenge.

In the present study, 23.9% of dogs relapsed 3–6 h after a food challenge and 60.9% in <12 h. The fact that recurrence took several hours may have been influenced by the transit time in the gastrointestinal tract. Orally administered antigens require a mean time of 3 h to reach the blood¹⁰ and thus affect organs other than the gastrointestinal system. By contrast, a previous study indicated that the duration to onset of clinical signs was a few days.¹¹ We also found that some dogs developed pruritus in this time frame. This duration corresponds with that of type IV hypersensitivity, which develops within a few hours to days by way of a cell-mediated immune response. This is supported by a previous study in which lymphocytes responding to food allergens were shown to be present in the blood of dogs with CAFR.¹² This illustrates that dogs should be observed for several days after starting the challenge test because CAFR may cause a delayed response owing to type IV hypersensitivity.

Our results provide new insights into food challenge testing in client-owned dogs and will assist veterinarians and owners in decision-making regarding food challenge tests.

References

- Olivry T, Mueller RS. Critically appraised topic on adverse food reactions of companion animals (3): prevalence of cutaneous adverse food reactions in dogs and cats. *BMC Vet Res* 2017; 13: 51.
- Mueller RS, Olivry T. Critically appraised topic on adverse food reactions of companion animals (4): can we diagnose adverse food reactions in dogs and cats with in vivo or in vitro tests? *BMC Vet Res* 2017; 13: 275.
- Taylor SL, Moneret-Vautrin DA, Crevel RWR et al. Threshold dose for peanut: risk characterization based upon diagnostic oral challenge of a series of 286 peanut-allergic individuals. *Food Chem Toxicol* 2010; 48: 814–819.
- Perry TT, Matsui EC, Conover-Walker MK et al. Risk of oral food challenges. *J Allergy Clin Immunol* 2004; 114: 1,164–1,168.
- Hensel P, Santoro D, Favrot C et al. Canine atopic dermatitis: detailed guidelines for diagnosis and allergen identification. *BMC Vet Res* 2015; 11: 196.
- Rybniček J, Lau-Gillard PJ, Harvey R et al. Further validation of a pruritus severity scale for use in dogs. *Vet Dermatol* 2009; 20: 115–122.
- Olivry T, Mueller RS. Critically appraised topic on adverse food reactions of companion animals (7): signalment and cutaneous manifestations of dogs and cats with adverse food reactions. *BMC Vet Res* 2019; 15: 140–146.
- Marsella R, Nicklin C, Lopez J. Studies on the role of routes of allergen exposure in high IgE-producing beagle dogs sensitized to house dust mites. *Vet Dermatol* 2006; 17: 306–312.
- Mueller RS, Unterer S. Adverse food reactions: pathogenesis, clinical signs, diagnosis and alternatives to elimination diets. *Vet J* 2018; 236: 89–95.
- Mizuta H, Kawazoe Y, Haga K et al. Determination of small intestinal transit time in beagle dogs using salicylazosulfapyridine. *Yakugaku Zasshi* 1989; 109: 760–765.
- Favrot C, Bizikova P, Fischer N et al. The usefulness of short-course prednisolone during the initial phase of an elimination diet trial in dogs with food-induced atopic dermatitis. *Vet Dermatol* 2019; 30: 498–e149.
- Kawano K, Oumi K, Ashida Y et al. The prevalence of dogs with lymphocyte proliferative responses to food allergens in canine allergic dermatitis. *Polish J Vet Sci* 2013; 16(2): 323–332.

Supporting Information

Table S1. List of the dogs enrolled in this study.

Additional Supporting Information may be found in the online version of this article.

Résumé

Contexte – Un régime d'éviction et un test de provocation alimentaire restent le gold standard pour le diagnostic des réactions cutanées liées à l'alimentation (CAFR) chez les chiens. Cependant, les tests alimentaires sont difficiles à réaliser compte tenu du manque d'informations précises sur les signes cliniques attendus et le temps au bout duquel les symptômes se développent après la provocation.

Objectifs – Décrire les résultats du test de provocation alimentaire effectué chez des chiens de propriétaires sous observation.

Matériels et méthodes – Une évaluation rétrospective des données médicales des chiens présentant du prurit a été réalisée. Les infections cutanées et/ou les parasites ont été traités avant inclusion dans l'étude. Les chiens qui ont reçu un régime d'éviction avec amélioration clinique et rechute avec test de provocation alimentaire ont été inclus dans l'étude. Un score d'une échelle visuelle analogue du prurit a été enregistré comme index de prurit avant et à la fin du régime d'éviction avant provocation. Le début du temps de récurrence des signes cliniques et les sites corporels atteints après la provocation alimentaire ont été enregistrés par les propriétaires.

Résultats – Nous examinons les données médicales de 310 chiens avec prurit dont 46 chiens avec un diagnostic de CAFR ont été inclus dans cette étude. Une amélioration clinique significative a été observée après le régime d'éviction ($P < 0.0001$). Tous les chiens présentaient du prurit et aucun n'a présenté d'anaphylaxie après les tests alimentaires. Parmi ces chiens, 60.9% ont développé du prurit dans les 12h suivant la provocation. Notamment, 23.9% des chiens ont développé un prurit dans les 3-6h. Les membres ont montré la plus haute fréquence de prurit (56.5%), suivi par la face (26.1%).

Conclusions et importance clinique – Cette étude est la première à décrire en détail le moment d'apparition des signes cliniques et des sites corporels atteints après provocation alimentaire. Ces résultats aideront les vétérinaires et les propriétaires de chiens dans l'interprétation des tests de provocation alimentaire.

RESUMEN

Introducción – las pruebas de dieta de eliminación y las pruebas de provocación alimentaria siguen siendo el modelo estándar para el diagnóstico de reacciones adversas alimentarias cutáneas (CAFR) en perros. Sin embargo, las pruebas de provocación alimentaria son difíciles de realizar debido a la falta de información definitiva sobre los signos clínicos esperados y el marco de tiempo dentro del cual se desarrollan después de la provocación.

Objetivos – Informar de los resultados de la prueba de provocación alimentaria realizada en perros de propietarios privados bajo observación.

Métodos y materiales – Se realizó una evaluación retrospectiva de los historiales clínicos de perros que presentaban prurito. Las infecciones de la piel y/o los parásitos se trataron antes de su inclusión en el estudio. En este estudio se incluyeron perros que se sometieron a una dieta de eliminación con mejoría clínica y recaída con pruebas de provocación alimentaria. Se determinó una puntuación de prurito en la escala análoga visual como índice de prurito antes y al final de la prueba de dieta de eliminación antes de la exposición. Los propietarios registraron el tiempo hasta la recaída de los signos clínicos y los sitios del cuerpo afectados después de la prueba de provocación alimentaria.

Resultados – Examinamos los historiales clínicos de 310 perros con prurito y de estos 46 perros con diagnóstico de CAFR se incluyeron en este estudio. Se observó una mejora clínica significativa después del ensayo de dieta de eliminación ($P < 0.0001$). Todos los perros mostraron prurito y ninguno presentó anafilaxia después de la prueba de provocación alimentaria. Entre estos perros, el 60,9% desarrolló prurito dentro de las 12 h posteriores a la provocación alimentaria. En particular, el 23,9% de los perros desarrollaron prurito en 3 a 6 h. Las extremidades presentaron la mayor frecuencia de prurito (56,5%), seguidas de la cara (26,1%).

Conclusiones y relevancia clínica – este estudio es el primero en informar detalles del tiempo hasta la aparición de los signos clínicos y zonas del cuerpo afectadas después de la prueba de provocación alimentaria en perros. Estos resultados ayudarán a los veterinarios y propietarios de perros en el proceso de toma de decisiones con respecto a las pruebas de provocación alimentaria.

Zusammenfassung

Hintergrund – Eliminationsdiäten und Futterprovokationstests bleiben der Goldene Standard zur Diagnose kutaner Futtermittelnebenwirkungen (CAFR). Futterprovokationstests sind allerdings schwierig durchzuführen, was darin begründet ist, dass ein Mangel an definitiver Information in Bezug auf die zu

erwartenden klinischen Zeichen besteht und auch des Zeitrahmens innerhalb welchem sie nach der Provokation entstehen.

Ziele – Das Ziel war ein Bericht über die Ergebnisse eines Futterprovokationstests, der bei privaten Hunden unter Beobachtung durchgeführt wurde.

Methoden und Materialien – Eine retrospektive Evaluierung der medizinischen Daten der Hunde, die mit Juckreiz vorgestellt wurden, wurde durchgeführt. Hautinfektionen und/oder Parasiten wurden vor Aufnahme in die Studie behandelt. Hunde, die sich mit einer durchgeführten Eliminationsdiät klinisch verbesserten und sich mit der Futterprovokation wieder verschlechterten, wurden in die Studie aufgenommen. Der Juckreiz wurde mittels Pruritus Visual Analog Skala als Index für den Juckreiz vor und am Ende der Eliminationsdiät, also vor der Provokation, bewertet. Der Zeitpunkt des Wiederauftretens der klinischen Zeichen und der betroffenen Körperstellen nach der Futterprovokation wurde von den BesitzerInnen festgehalten.

Ergebnisse – Wir untersuchten die medizinischen Daten von 310 Hunden mit Juckreiz und von diesen wurden 46 Hunde mit der Diagnose einer CAFR in die Studie aufgenommen. Nach der Eliminationsdiät wurde eine signifikante klinische Verbesserung beobachtet ($P < 0,0001$). Alle Hunde zeigten Juckreiz und keiner zeigte nach der Futterprovokation eine Anaphylaxie. Von diesen Hunden entwickelten 60,9% Juckreiz innerhalb von 12 h nach der Futterprovokation. Bemerkenswerterweise entwickelten 23,9% der Hunde den Juckreiz innerhalb von 3-6 h. An den Extremitäten zeigte sich die deutlichste Juckreizfrequenz (56,5%), gefolgt vom Gesicht (26,1%).

Schlussfolgerungen und klinische Bedeutung – Es handelt sich hierbei um die erste Studie, die Details über den Zeitpunkt berichtet, ab dem sich nach einer Futterprovokation klinische Zeichen zeigen und welche Körperstellen betroffen sind. Diese Ergebnisse werden TierärztInnen und HundebesitzerInnen beim Entscheidungsprozess in Bezug auf Futterprovokationstests helfen.

要約

背景 – 犬の皮膚食物有害反応 (CAFR) を診断する上で、除去食試験や食物負荷試験は依然としてゴールドスタンダードである。しかし、食物負荷試験は、予想される臨床症状や、負荷後に発症する時間帯などの明確な情報がないため、実施が困難である。

目的 – 本研究の目的は、観察中のクライアント所有犬に実施した食物負荷試験の結果を報告することであった。

材料と方法 – みを呈した犬の医療記録の回顧的な評価を行った。皮膚感染症および/または寄生虫感染は、研究に含める前に治療された。除去食試験を行い、臨床的に改善し、食物負荷試験で再燃した犬を本研究に組み入れた。負荷前の除去食試験の前と終了時に、痒みの指標として Visual Analog Scale スコアを記録した。食物負荷試験後の臨床症状の再発までの時間および罹患部位を飼い主が記録した。

結果 – 痒みを呈した犬310頭の医療記録を調査し、そのうちCAFRと診断された46頭が本研究に登録された。除去食試験後、有意な臨床的改善が認められた ($P < 0.0001$)。すべての犬が痒みを呈し、食物負荷試験後にアナフィラキシーを呈した犬はいなかった。これらの犬のうち、60.9%が食物負荷から12時間以内に痒みを発症した。特に、23.9%の犬が3~6時間以内に掻痒症を発症した。掻痒症の頻度は、四肢が最も高く (56.5%)、次いで顔面 (26.1%) であった。

結論と臨床的妥当性 – 本研究は、犬の食物負荷試験後の臨床症状の発症時間および罹患部位の詳細を報告した初めての研究である。これらの結果は、獣医師や犬の飼い主が食物負荷試験に関する意思決定を行う際の助けとなるだろう。

背景 – 食物排除試験と食物刺激試験仍然是诊断犬皮肤不良食物反应(CAFR)的金标准。然而,在激发试验后,由于预期临床症状和发作时间信息不明确,因此难以进行食物激发试验。

目的 – 报告进行食物激发试验的私家犬的观察结果。

方法和材料 – 对瘙痒犬的病历进行回顾性评价。在入组研究前,治疗皮肤感染和/或寄生虫。本研究纳入了接受食物排除试验得到临床改善,并且食物激发试验复发的犬。用瘙痒视觉模拟量表评分,记录食物排除试验前,以及试验结束时在激发试验前的瘙痒指数。犬主人记录了临床症状复发的时间,以及食物激发试验后的身体发病部位。

结果 – 我们检查了310只瘙痒犬的病历,其中诊断为CAFR的46只犬纳入组本研究。食物排除试验后观察到显著的临床改善($P < 0.0001$)。在食物激发试验后,所有犬均表现出瘙痒,均未出现过敏性休克。在这些犬中,60.9%在食物激发后12 h内出现瘙痒。值得注意的是,23.9%的犬在3-6h内出现瘙痒。瘙痒在四肢出现频率最高 (56.5%),其次是面部(26.1%)。

结论和临床相关性 – 本研究首次报告了犬食物激发试验后,临床症状发作时间和身体发病部位的详细信息。这些结果将帮助兽医和犬主人做出关于食物激发试验的决策。

Resumo

Contexto – As dietas de eliminação e o desafio dietético continuam sendo o padrão ouro para o diagnóstico de reações adversas a alimentos (CAFRs) em cães. Entretanto, o desafio dietético é complicado de se realizar devido à falta de informações definitivas sobre os sinais clínicos esperados e em quanto tempo eles se desenvolvem após o desafio.

Objetivos – Relatar os resultados do desafio dietético realizado em cães de clientes sob observação.

Métodos e materiais – Realizou-se um estudo retrospectivo dos prontuários de cães apresentando prurido. Infecções cutâneas e/ou parasitas foram tratados antes da inclusão no estudo. Os cães que apresentaram melhora com a dieta de eliminação e piora após o desafio dietético foram incluídos no estudo. O escore da escala analógica visual de prurido (PVAS) foi registrado como um índice de prurido antes e ao final da dieta de eliminação antes do desafio. O tempo para a recidiva dos sinais clínicos e as áreas corpóreas afetadas após o desafio dietético foram registrados pelos proprietários.

Resultados – Os prontuários de 310 cães com prurido foram examinados e 46 destes cães com diagnóstico de CAFR foram incluídos no estudo. Observou-se melhora significativa após a dieta de eliminação ($P < 0.0001$). Todos os cães exibiram prurido e nenhum apresentou anafilaxia após o desafio dietético. Dentre esses cães, 60,9% desenvolveram prurido dentro de 12 h após o desafio dietético. Vale destacar que 23,9% dos cães apresentaram prurido dentro de 3-6 h. Os membros exibiram a maior frequência de prurido (56,5%), seguido da face (26,1%).

Conclusões e relevância clínica – Este estudo foi o primeiro a detalhar o tempo de surgimento dos sinais clínicos e as áreas corpóreas afetadas depois do desafio dietético em cães. Estes resultados vão auxiliar os veterinários e proprietários no processo de tomada de decisão em relação ao desafio dietético.

Supplementary Table 1. List of the dogs which were enrolled in this study.

No.	Protein source of elimination diet	Sex	Body weight (kg)	Age (year-old)	Breed	PVAS score (Pre)	PVAS score (Post)	Time of onset (h)	Lesion												
									Eyelid	Lips	Face	Ear pinnae	Limbs	Chest and neck	Axillae	Abdomen	Groin	Back and dorsolumbar area	Femur	Margin of anus	Tail
1	Salmon	Male	5	7	Miniature dachshund	6	2	12					○				○				
2	Salmon	Female	30.3	3	Pitbull	8	2	4	○	○	○	○	○	○				○			
3	Salmon	Male	6	6	French bulldog	6	1	8	○				○	○							
4	Salmon	Male	5.5	10	Toy poodle	8	1	6					○	○							
5	Salmon	Male	5.8	13	Toy poodle	5	1	1.5	○		○										
6	Salmon	Male	5	5	Toy poodle	4	0	6											○		
7	Salmon	Female	9.7	4	French bulldog	6	2	12			○	○	○								
8	Salmon	Female	8.6	5	French bulldog	7	1	4			○		○			○					
9	Salmon	Male	2.5	1	Papillon	5	0	7	○			○	○								
10	Salmon	Male	5	5	Toy poodle	10	0	12				○	○								
11	Salmon	Male	2.1	1	Toy poodle	7	1	5		○				○							○
12	Salmon	Male	16.7	12	Australian chelpy	8	0	240							○						
13	Salmon	Male	8.1	10	Cairn terrier	10	1	12		○											
14	Salmon	Male	4.3	11	Miniature dachshund	8	1	3			○					○					
15	Salmon	Male	1.9	3	Toy poodle	6	2	3.5					○								
16	Salmon	Female	2.7	7	Miniature pinscher	9	3	3			○		○	○							
17	Salmon	Female	7.3	2	French bulldog	5	1	6			○										
18	Salmon	Female	28	7	Golden retriever	8	0	24				○									
19	Salmon	Female	4.8	5	Pomeranian	5	1	120					○								
20	Salmon	Male	5	7	Pug	5	0	4						○							
21	Salmon	Male	23	5	Toy poodle	6	2	24					○								
22	Salmon	Male	5.4	5	Boston terrier	8	2	12	○												
23	Salmon	Male	6	12	Maltese	6	0	24	○				○								
24	Horseflesh	Male	4.2	4	Toy poodle	8	1	10		○		○	○								
25	Horseflesh	Male	3.6	10	Toy poodle	7	3	72				○	○			○					
26	Horseflesh	Male	7.3	5	Pug	7	1	18		○											
27	Horseflesh	Male	4.1	1	Toy poodle	5	1	72						○							
28	Horseflesh	Male	4.5	4	Toy poodle	10	1	5				○	○								
29	Horseflesh	Male	6.8	5	Toy poodle	6	1	24					○								
30	Horseflesh	Male	3	7	Bichon frise	8	1	12		○											
31	Horseflesh	Female	26	1	Golden retriever	5	1	22					○				○		○		
32	Horseflesh	Female	7.3	4	Jack russell terrier	8	1	18		○											
33	Horseflesh	Female	9.9	6	French bulldog	8	1	120					○								
34	Horseflesh	Female	4	6	Yorkshire terrier	2	0	2									○				
35	Horseflesh	Male	28	2	Airedale terrier	5	1	36								○					
36	Horseflesh	Male	3.8	2	Chihuahua	4	1	24					○								
37	Horseflesh	Female	4.7	6	Toy poodle	4	1	12	○	○	○										
38	Horseflesh	Male	5.4	4	Shih tzu	8	1	4					○								
39	Horseflesh	Female	4.3	4.5	Toy poodle	8	0	6		○											
40	Horseflesh	Female	3.1	2	Toy poodle	8	1	60			○										
41	Horseflesh	Male	39	9	Labrador retriever	9	2	72			○		○								
42	Horseflesh	Female	6	7	Mixed breed	8	0	2					○						○		
43	Horseflesh	Female	7.7	8	Shiba Inu	8	1	2	○		○										
44	Horseflesh	Male	8.3	12	Bichon frise	8	0	24					○								
45	Horseflesh	Male	4.5	8	Mixed breed	5	1	12			○		○						○		
46	Horseflesh	Male	4.5	3	Miniature dachshund	5	2	24					○								