

Serum IgE against cross-reactive carbohydrate determinants (CCD) in healthy and atopic dogs

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Background – Cross-reactive carbohydrate determinants (CCD) are Immunoglobulin E (IgE)-binding carbohydrate structures common to plant and insect species. In people, anti-CCD IgE is thought to be clinically irrelevant, but to have the potential to confound serological IgE test interpretations. Previous studies reported the detection of anti-CCD IgE in 24–73% of atopic dog sera; prevalence in healthy dogs has not been reported.

Objective – To compare the prevalence of anti-CCD IgE in a group of healthy and atopic dogs.

Animals – Sera from 61 healthy dogs and 101 dogs with a clinical diagnosis of atopic dermatitis were analyzed for IgE against CCD.

Methods and materials – Sera were analyzed for the presence of anti-CCD IgE and IgE against environmental allergens using a commercial multiplex allergen-specific IgE assay.

Results – Anti-CCD IgE was detected in 17 of 101 (16.8%) atopic dog sera and eight of 61 (13.1%) healthy dog sera ($P = 0.65$, Fisher's exact test). All healthy and atopic dogs with anti-CCD IgE had strong reactivity to grass pollens.

Conclusions and clinical importance – A similar prevalence of anti-CCD IgE was found in healthy and atopic dogs. Further investigation is warranted to determine the clinical significance of anti-CCD IgE antibodies in dogs, how they are best detected and if blocking these antibodies during diagnostic testing has clinical value.

Introduction

Commercial serum allergen-specific Immunoglobulin E (IgE) tests are commonly used in veterinary medicine for formulation of allergen immunotherapy prescriptions for dogs with a clinical diagnosis of atopic dermatitis (AD). Therapeutic challenges arise when these *in vitro* tests provide results that are inconsistent with a dog's history or clinical signs. For example, it may be difficult to interpret results from dogs with strong positive reactions to allergens in a season during which the dog is not symptomatic.

A factor that could be affecting test results is IgE reactivity against highly antigenic, carbohydrate structures commonly found on plant and insect allergens. These structures are known as cross-reactive carbohydrate determinants (CCD).^{1–3} In people, CCDs have been identified as a source of cross-reactivity in IgE serological tests,

leading to positive test reactions that are not related to clinical signs.^{4–7}

In the veterinary literature, there are three studies evaluating anti-CCD IgE in atopic dogs. In two published studies, 23 and 39% of serum samples from atopic dogs were positive for anti-CCD IgE antibodies, respectively.^{8,9} In an additional published abstract, a prevalence of 73% was reported.¹⁰ The authors are unaware of any published studies investigating the prevalence of anti-CCD IgE in healthy dogs.

The goal of the present study was to compare the prevalence of anti-CCD IgE in healthy and atopic dogs from the same geographical region using a commercial *in vitro* diagnostic test.

Methods and materials

Serum samples

Atopic dogs

Serum samples were obtained from a frozen serum bank maintained at the authors' institution. All dogs with AD undergoing intradermal and/or serum IgE testing routinely had serum stored in the bank for research purposes. Dog owners signed a consent and release form allowing use of their animal's serum for future research. Serum samples selected for inclusion in this study were from dogs with a clinical diagnosis of AD (history and clinical signs consistent with AD, fulfillment of at least five published criteria, and elimination of parasitic and infectious causes of pruritus), regardless of results of intradermal or serological tests.¹¹ Serum was collected between January 2014 and December 2017.

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Conflicts of Interest: Douglas DeBoer supplies test kit components to Biocheck GmbH. No other conflicts of interest have been declared.

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Healthy dogs

Samples from healthy dogs were obtained from the authors' veterinary teaching hospital as surplus serum left from healthy-pet test requests (i.e. heartworm testing, annual wellness screening). Medical records were reviewed to exclude dogs with any history of dermatological or systemic illness.

Assay for IgE against CCD and environmental allergens

Sera from both healthy and atopic dogs were analyzed for presence of anti-CCD IgE, as well as IgE against a panel of environmental allergens, using a commercially available allergen-specific IgE assay kit, according to the manufacturer's instructions (Polycheck Canis 20, Biocheck GmbH; Muenster, Germany), performed in the authors' laboratory. The test consists of allergen extracts printed as individual lines on a membrane strip. The strip is incubated sequentially with patient serum, biotinylated anti-canine IgE, streptavidin-alkaline phosphatase and substrate solution. Colour development was measured using a flatbed scanner and image analysis software provided by the manufacturer. Results were expressed in arbitrary units/mL (U/mL) based upon a standard curve generated from five reference standard lines on each strip. A positive result was defined based on the manufacturer's defined positive threshold of greater than 0.15 U/mL.

Statistical analyses

Fisher's exact test was used to compare the proportion of anti-CCD IgE-positive to IgE-negative samples in both groups in a 2 × 2 contingency table. The Wilcoxon–Mann–Whitney U-test was used to compare the concentrations of anti-CCD IgE between atopic and healthy dogs. Statistical analysis of data was performed with PRISM 6 (GraphPad Software Inc.; La Jolla, CA, USA).

Results

Proportion of dogs positive for anti-CCD IgE

Table 1 shows the number of healthy and atopic dogs with anti-CCD IgE. Seventeen of 101 atopic dogs (16.8%) had measurable anti-CCD IgE (>0.15 U/mL) and eight of 61 healthy dogs (13%) had anti-CCD IgE ($P = 0.65$, Fisher's exact test).

Concentration of anti-CCD IgE in CCD-positive dogs

The concentrations of anti-CCD IgE in the CCD-positive samples are shown in Figure 1. The mean anti-CCD IgE concentration in CCD-positive healthy and atopic dogs was 0.38 U/mL (range, 0.16–9.5 U/mL) and 2.4 U/mL (range, 0.17–9.3 U/mL), respectively. ($P = 0.04$, Wilcoxon–Mann–Whitney U-test).

Presence of anti-CCD IgE in relation to other positive reactions on the panel

The Polycheck assay strip contains individual environmental allergens belonging to six categories (tree pollens, weed pollens, grass pollens, fungi, mites and fleas). When each dog's anti-CCD IgE test result was examined individually, relative to these categories the following

Table 1. Numbers of healthy and atopic dogs with positive or negative serum CCD-specific IgE (positive: 0.15 U/mL or greater)

	Anti-CCD IgE Positive	Anti-CCD IgE Negative
Atopic (n = 101)	17 (16.8%)	84 (83.1%)
Healthy (n = 61)	8 (13.1%)	53 (86.8%)

There was no significant difference between the proportions of anti-CCD IgE positive healthy and atopic dogs ($P = 0.65$, Fisher's exact test).

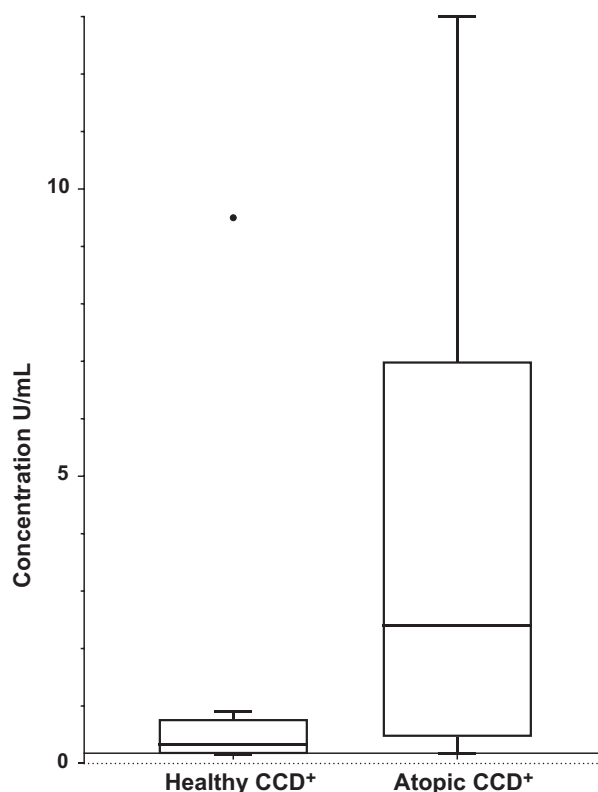


Figure 1. Box plot of anti-CCD IgE serum concentrations in healthy and atopic dogs as determined by the Polycheck assay.

Central line indicates median; upper and lower box lines indicate 75th and 25th percentiles; outer "whiskers" indicate minimum and maximum. The separately plotted data point located outside the fences ("whiskers") indicates an outlier for the healthy group of anti-CCD IgE-positive dogs. Concentrations of anti-CCD IgE are expressed in U/mL. The healthy dog group had significantly lower concentrations than the atopic dog group ($P = 0.04$, Wilcoxon–Mann–Whitney U-test).

observations were made (Figure 2 and Table 2): (i) all dogs with anti-CCD IgE, whether healthy or atopic, had a positive result to at least one grass pollen and one environmental mite; (ii) dogs with anti-CCD IgE, whether healthy or atopic, were more likely to have a positive result to at least one weed pollen, as compared to CCD-negative dogs. ($P < 0.01$, Fisher's exact test); (iii) dogs with anti-CCD IgE, whether healthy or atopic, were more likely to have a positive result to at least one tree pollen, as compared to CCD-negative dogs. ($P < 0.01$, Fisher's exact test); and (iv) the number of positive reactions to fungi was relatively low and did not vary between CCD IgE-positive and IgE-negative samples. ($P = 0.14$, Fisher's exact test).

In addition, the majority of samples, whether from healthy or atopic dogs, were positive to mites (135 of 162 samples) and to flea (129 of 162 samples).

Discussion

The present study investigated the presence of anti-CCD IgE in 61 healthy dogs and 101 atopic dogs using a commercially available allergen-specific IgE assay. It was demonstrated that both healthy and atopic dogs can have anti-CCD IgE, although healthy dogs generally have lower

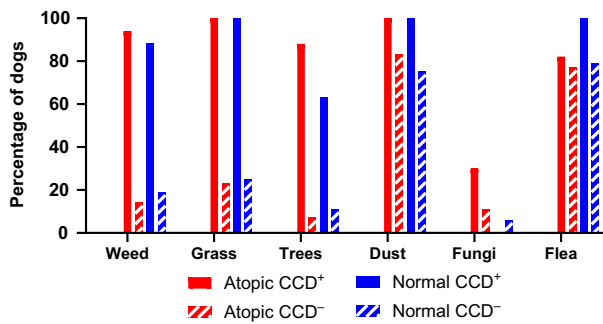


Figure 2. Percentage of anti-CCD IgE-positive and IgE-negative dogs with a positive reaction to at least one allergen on Polycheck assay from each category: weed, grass, tree, mites, fungi and flea.

concentrations. It also was found that dogs positive for CCD IgE are highly likely to have positive results for pollen allergen-specific IgE.

There are three previous studies evaluating the presence of anti-CCD IgE in atopic dogs. In the first study, which also used the Polycheck assay, the investigators reported the prevalence of anti-CCD IgE in a small group of atopic dogs in the USA and found that 9 of 38 (24%) were positive.⁸ As in the present study, the investigators also found that the presence of anti-CCD IgE was associated with positive reactions to pollen allergens. There was no healthy dog population for comparison. In a second unpublished report,¹⁰ using a different assay, investigators reported a prevalence of anti-CCD IgE of 73% in 95 atopic dogs living in the UK. In addition, they also noted that positive serum samples reactive to more than three grass pollens also were reactive to CCD.¹⁰ A third study of atopic dogs from Germany, using a third and different assay system, reported a CCD IgE-positive prevalence of 39%.⁹ Differences in reported prevalence might easily be explained by differences in patient inclusion criteria, in the assay systems used and perhaps even by regional variation. However, a clear and consistent result, also part of the findings herein, is demonstration that positive anti-CCD IgE in a serum sample from an atopic dog always is associated with positive reactions to pollen allergens. Moreover, the present results extend this observation to healthy dog samples, in addition to those from atopic dogs.

Studies in people report the prevalence of anti-CCD IgE to vary from 18% to 71% in allergic patients.^{1,4,12,13} The consensus among physician allergists is that anti-CCD IgE antibodies are not biologically active; in other words, they do not influence clinical symptoms.^{5,7} The proposed

mechanism for the absence of biological activity in IgE associated with these glycoproteins is based on the monovalent nature of the CCD structure, making it incapable of cross-linking IgE bound to mast cells.^{1,3,14} This fact not only limits biological activity, but would also yield a negative intradermal test result. However, the IgE is fully able to bind to monovalent CCD in a serological assay, resulting in a positive, yet probably irrelevant, result. In people, anti-CCD IgE antibodies in pollen allergic patients can produce confounding results on serological testing, including marked discrepancies with results of intradermal tests.^{4,12} Initial evidence also suggests that this is true in dogs: using a special blocking step to eliminate anti-CCD IgE reactivity in a serological assay leads to better correlation of serology results with those of intradermal testing.⁹

When actual concentrations of anti-CCD IgE were examined in atopic versus healthy dogs, it was found that atopic dogs had higher concentrations. This would likely be explained by the fact that atopic dogs are genetically programmed to have immunological responses that are Th2-biased and therefore may have overall higher IgE responses.¹⁵

A limitation of the present study is that it is retrospective and therefore cannot fully capture the entire health history of the patients evaluated. For example, it is conceivable that some of our healthy dogs with positive test results were truly early atopic dogs and clinical signs were not yet evident.

Of additional note is that nearly all dogs, whether healthy or atopic, were found to have positive reactions to both environmental mites and fleas. This result underscores the importance of proper diagnostic evaluation before serological testing and never using serology as a tool to initially diagnose allergy.

Treatment of AD in dogs with allergen immunotherapy is commonly determined based upon positive results of intradermal tests or allergen-specific IgE serology. Due to the similar prevalence of anti-CCD IgE in atopic and healthy dogs, along with strong association with positive pollen reactions on serological testing, the results of the present study, along with those of others, suggest that serological testing results can be adversely affected by anti-CCD IgE binding to glycoproteins on pollen allergens. Additionally, the finding of anti-CCD IgE in some healthy dogs, with no clinical signs, provides additional evidence that it may be biologically irrelevant.

In conclusion, the present study found that anti-CCD IgE antibodies are present in both healthy and atopic

Table 2. Number of anti-CCD IgE positive and negative healthy and atopic dogs with a positive reaction to at least one allergen on the Polycheck assay

Test group	All dogs		Atopic dogs		Healthy dogs	
	CCD+	CCD–	CCD+	CCD–	CCD+	CCD–
Weed	23 of 25	22 of 137	16 of 17	12 of 84	7 of 8	10 of 53
Grass	25 of 25	32 of 137	17 of 17	19 of 84	8 of 8	13 of 53
Tree	20 of 25	12 of 137	15 of 17	6 of 84	5 of 8	6 of 53
Mite	25 of 25	110 of 137	17 of 17	70 of 84	8 of 8	40 of 53
Fungi	5 of 25	12 of 137	5 of 17	9 of 84	0 of 8	3 of 53
Flea	22 of 25	107 of 137	14 of 17	65 of 84	8 of 8	42 of 53

Allergens were divided into six categories: weed, grass, tree, mites, fungi and fleas.

dogs, albeit in differing concentrations. In addition, there was a strong association of anti-CCD IgE and positive reactions to pollen allergens, regardless of the dog's status as healthy or atopic. Blocking anti-CCD IgE before serological testing could increase specificity of allergen-specific IgE tests, leading to more clinically representative results. Increased accuracy could lead to better-targeted immunotherapy formulations and greater efficacy of immunotherapy in canine patients. Future studies are needed to determine the clinical significance of anti-CCD IgE antibodies in dogs, how they are best detected and if blocking these antibodies during diagnostic testing has clinical value.

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Résumé

Contexte – Les CDD (cross-reactive carbohydrate determinants) sont des carbohydrates porteurs d'immunoglobulines E (IgE) fréquents pour les plantes et les insectes. Chez l'homme, les IgE anti-CDD sont considérés comme non significatifs cliniquement mais ayant un potentiel de confusion d'interprétation des tests sérologiques d'IgE. Les études précédentes rapportent la détection des IgE anti-CDD dans 24-73% des sera des chiens atopiques ; la prévalence chez le chien sain n'a pas été décrite.

Objectif – Comparer la prévalence des IgE anti-CDD dans un groupe de chiens sains et atopiques.

Sujets – Les sera de 61 chiens sains et 101 chiens atteints de dermatite atopique ont été analysés pour les IgE anti CDD.

Matériels et méthodes – Les sera ont été analysés pour la présence des IgE anti-CDD et les IgE dirigés contre les allergènes environnementaux à l'aide d'un test commercial multiplex d'IgE spécifique d'allergènes.

Résultats – Les IgE anti-CDD étaient détectés dans 17 des 101 (16,8%) sera de chiens atopiques et huit des 61 (13,1%) sera des chiens sains ($P = 0.65$, Fisher's exact test). Tous les chiens sains et atopiques avec IgE anti-CDD avaient une forte réactivité aux pollens d'herbe.

Conclusions et importance clinique – Une prévalence identique d'IgE anti-CDD a été trouvée chez les chiens sains et atopiques. Davantage d'études sont nécessaires pour déterminer la signification clinique des anticorps IgE anti-CDD chez le chien, comment les détecter au mieux et si leur blocage au cours des tests diagnostics ont une valeur clinique.

Resumen

Introducción – los determinantes de carbohidratos con reactividad cruzada (CCD) son estructuras de carbohidratos que se unen a la inmunoglobulina E (IgE) comunes a especies de plantas e insectos. En las personas, se cree que la IgE anti-CCD es clínicamente irrelevante, pero tiene el potencial de confundir las interpretaciones de la prueba de IgE serológica. Estudios anteriores informaron de la detección de IgE anti-CCD en un 24 a 73% de los sueros atópicos para perros; no se ha publicado la prevalencia en perros sanos.

Objetivo – comparar la prevalencia de IgE anti-CCD en un grupo de perros sanos y atópicos.

Animales – se analizaron los sueros de 61 perros sanos y 101 perros con diagnóstico clínico de dermatitis atópica para detectar IgE contra CCD.

Métodos y materiales – se analizaron los sueros para determinar la presencia de IgE e IgE anti-CCD contra alérgenos ambientales usando un ensayo comercial de IgE multiplex específico para alérgenos.

Resultados – se detectó IgE anti-CCD en 17 de 101 (16,8%) de sueros de perros atópicos y ocho de 61 (13,1%) sueros de perros sanos ($P = 0,65$, prueba exacta de Fisher). Todos los perros sanos y atópicos con IgE anti-CCD tuvieron una fuerte reactividad a los pólenes de hierba.

Conclusiones e importancia clínica – se encontró una prevalencia similar de IgE anti-CCD en perros sanos y atópicos. Se justifica una investigación adicional para determinar la importancia clínica de los anticuerpos IgE anti-CCD en perros, cómo se detectan mejor y si el bloqueo de estos anticuerpos durante las pruebas de diagnóstico tiene valor clínico.

Zusammenfassung

Hintergrund – Kreuz-reaktive Kohlenhydrat Determinanten (CCD) sind Immunglobulin E (IgE)- bindende Kohlenhydrat Strukturen, die für Pflanzen und Insekten Spezies typisch sind. Beim Menschen hält man anti-CCD IgE für klinisch irrelevant, aber man geht davon aus, dass sie das Potential haben, die serologischen IgE Interpretationen durcheinander zu bringen. Frühere Studien berichteten vom Auftreten von anti-CCD IgE bei 24-73% der atopischen Sera von Hunden; die Prävalenz bei gesunden Hunden wurde bisher nicht publiziert.

Ziel – Ein Vergleich der Prävalenz von anti-CCD IgE in einer Gruppe gesunder und atopischer Hunde.

Tiere – Sera von 61 gesunden Hunden und von 101 Hunden mit der klinischen Diagnose einer atopischen Dermatitis wurde auf IgE gegen CCD analysiert.

Methoden und Materialien – Es wurden Sera auf das Vorkommen von anti-CCD IgE und IgE gegen Umweltallergene mittels kommerziellem Multiplex Allergen-spezifischem IgE Assay getestet.

Ergebnisse – Anti-CCD IgE wurde bei 17 von 101 (16,8%) Sera von atopischen Hunden und bei acht von 61 (13,1%) Sera von gesunden Hunden ($P = 0,65$; Fisher's Exact Test) gefunden. Alle gesunden und atopischen Hunde mit anti-CCD IgE zeigten eine stark positive Reaktivität zu Graspollen.

Schlussfolgerungen und klinische Bedeutung – Eine ähnliche Prävalenz von anti-CCD IgE wurde bei gesunden und atopischen Hunden gefunden. Weitere Untersuchungen sind nötig, um die klinische Signifikanz von anti-CCD IgE Antikörpern bei Hunden zu bestimmen, wie sie am besten bestimmt werden und ob ein Blockieren dieser Antikörper während dem diagnostischen Test von klinischem Wert ist.

要約

背景 – 交差反応性炭水化合物決定因子(CCD)は、植物および昆虫種に共通の免疫グロブリンE(IgE)結合炭水化合物構造物である。人においては、抗CCD IgEは臨床的に無関係であると考えられているが、血清学的IgE検査の解釈を混乱させる可能性があると考えられている。既存の研究では、アトピー犬血清の24～73%で抗CCD IgEが検出されたことが報告されている。健康犬の有病率は報告されていない。

目的 – 本研究の目的は、健康犬群とアトピー犬群における抗CCD IgEの有病率を比較することである。

被験動物 – 健康犬61頭およびアトピー性皮膚炎の臨床診断を受けた犬101頭の血清を、CCDに対するIgEについて解析した。

材料と方法 – 市販の多重アレルギー特異的IgEアッセイを用いて、血清を抗CCD IgEの存在および環境アレルギーに対するIgEについて解析した。

結果 – 抗CCD IgEは、101頭中17頭(16.8%)のアトピー犬血清と61頭中8頭(13.1%)の健康犬血清で検出された($P = 0.65$ 、フィッシャーの正確検定)。抗CCD IgEを有した健康犬およびアトピー犬はすべて、イネ科植物の花粉に対して強い反応性を示した。

結論と臨床的重要性 – 健康犬およびアトピー犬において、類似した抗CCD IgE有病率を検出した。犬における抗CCD IgE抗体の臨床的意義、それらの最良の検出方法、および診断検査中にこれらの抗体をブロッキングすることが臨床的価値を有するかどうかを判断するにはさらなる調査が必要である。

摘要

背景 – 交叉反应性碳水化合物决定簇(CCD)是植物和昆虫常见的免疫球蛋白E(IgE)结合碳水化合物的结构。人们认为抗CCD IgE在临床上无关紧要,但有可能扰乱对血清学IgE检测的判读。先前的研究报道了在24-73%的异位性犬血清中检测到了抗CCD IgE;而健康犬的检出率尚未见报道。

目的 – 比较一组健康和异位性犬中抗CCD IgE的检出率。

动物 – 分析61例健康犬和101例临床诊断为异位性皮炎犬的血清抗CCD的IgE。

方法和材料 – 使用多种商业化过敏原特异性IgE测定法,检测血清中抗CCD IgE和环境过敏原IgE的存在。

结果 – 在101例异位性犬血清中有17例(16.8%)抗CCD IgE;61例(13.1%)健康犬血清中有8例检测到了抗CCD IgE($P = 0.65$, Fisher精确检验)。所有具有抗CCD IgE的健康和异位性犬对草花粉都有较强的反应性。

结论和临床意义 – 在健康和异位性犬中发现了类似的抗CCD IgE检出率。有必要进行进一步的研究,以确定抗CCD IgE抗体在犬体内的临床意义,如何最好地检测这些抗体,以及在诊断试验中阻断这些抗体是否具有临床价值。

Resumo

Contexto – Os determinantes de carboidratos de reação cruzada (CCD) são estruturas de carboidratos que se ligam à imunoglobulina E (IgE), comuns a espécies de plantas e insetos. Em humanos, acredita-se que a IgE anti-CCD seja clinicamente irrelevante, mas possui o potencial de confundir interpretações do teste sorológico de IgE. Estudos anteriores relataram a detecção de IgE anti-CCD em 24-73% dos soros de cães atópicos; a prevalência em cães saudáveis não foi relatada.

Objetivo – Comparar a prevalência de IgE anti-CCD em um grupo de cães saudáveis e atópicos.

Animais – Os soros de 61 cães saudáveis e 101 cães com diagnóstico clínico de dermatite atópica foram pesquisados para a presença de IgE contra CCD.

Métodos e materiais – Os soros foram analisados quanto à presença de anti-CCD IgE e IgE contra alérgenos ambientais utilizando um ensaio multiplex comercial de IgE alérgeno-específico.

Resultados – A IgE anti-CCD foi detectada em 17 dos 101 (16,8%) soros de cães atópicos e oito dos 61 (13,1%) soros de cães saudáveis ($P = 0,65$, teste exato de Fisher). Todos os cães saudáveis e atópicos que tiveram IgE anti-CCD apresentaram forte reatividade ao pólen da gramíneas.

Conclusões e importância clínica – Observou-se uma prevalência semelhante de IgE anti-CCD em cães saudáveis e atópicos. Uma investigação mais aprofundada é necessária para determinar o significado clínico dos anticorpos IgE anti-CCD em cães, sua melhor forma de detecção e valor clínico do bloqueio desses anticorpos durante o teste diagnóstico.