

A preliminary study of serum IgE against cross-reactive carbohydrate determinants (CCD) in client-owned atopic dogs

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Background – Cross-reactive carbohydrate determinants (CCD) are defined carbohydrate portions of glycoprotein cell surface molecules common to many plant and insect species. Mammalian species recognize CCD as foreign antigens and can mount humoral immune responses against them. Approximately 20–37% of grass and venom allergic people possess circulating IgE against CCD; these antibodies are generally considered clinically irrelevant. Anti-CCD IgE is, however, recognized as a cause of false positive, clinically incongruent serum allergen test results in people; this phenomenon has not been investigated in animals.

Objective – To determine if anti-CCD IgE could be detected in sera of client-owned atopic dogs and how frequently it is found.

Animals – Sera from 38 dogs with a clinical diagnosis of atopic dermatitis and prior serological evidence of IgE antibodies, defined as a positive result to at least one mite and pollen (of any type).

Methods – Sera were analysed for IgE against CCD and environmental allergens with a commercially available multiplex enzyme-labelled allergen-specific IgE assay.

Results – Anti-CCD IgE was detected in nine of 38 (24%) of atopic dog sera. As with their human counterparts, all dogs with anti-CCD IgE had strong serological reactivity to grass pollens.

Conclusions and Clinical Importance – Anti-CCD IgE can confound serological allergen testing in people; the same might be true in dogs. Further studies are warranted to investigate the clinical implications of anti-CCD IgE in dogs, including the potential for these antibodies to affect serum allergen-specific IgE assays used for clinical diagnosis, and whether they are relevant to clinical disease.

Introduction

The presence of serum IgE against protein-linked carbohydrate epitopes is a documented cause of false positive reactions for *in vitro* allergen tests in people.^{1–5} These IgE-binding carbohydrates, known as cross-reactive carbohydrate determinants (CCDs), are abundant within the majority of allergenic plant and insect species; absent from mammalian tissue, they are strongly antigenic. Among pollen allergic humans, the prevalence of anti-CCD IgE is approximately 22% and increases proportionally as the number of pollen allergies increases.^{1,2} In most cases, immunological reactions to CCDs are clinically irrelevant, in that they do not lead to clinical symptoms.

Thus, the clinical relevance of anti-CCD IgE remains tied to its effect on *in vitro* diagnostic sensitivity, which can be significant in this subset of individuals. When physicians are faced with incongruent patient skin prick (or anamnesis) and serum test results, CCD has been identified as an important underlying cause for false positive reactions.^{3–5}

Similar diagnostic inconsistencies are regularly encountered in veterinary medicine, specifically with multi-allergen serum allergen panels. To the best of the authors' knowledge, no prior investigations had been conducted into the detectability or prevalence of anti-CCD IgE in dogs. The aim of this investigation was to determine if anti-CCD IgE was present and detectable in a small set of clinically atopic dogs and if so, with what overall frequency. We also sought to make observations in anti-CCD IgE-positive dogs as to how their overall test results compared to those of negative dogs (for example in number, type and strength of reactions).

Materials and Methods

Sera from dogs with a clinical diagnosis of atopic diagnosis were obtained from a frozen bank maintained at our institution. The main criteria for a sample to be included in the study was for it to have been taken from an atopic dog with prior serological evidence of sensitization to mites and pollens. More specifically, to

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qualify for inclusion, the dog must have: had a clinical diagnosis of atopic dermatitis (AD) as assessed by published criteria⁶ and other clinical historical and physical characteristics, and elimination of other causes of pruritic disease such as infections and parasitism; and undergone serological testing with a commercial allergen-specific IgE panel at the time (Heska Allercept panel, Heska Inc.; Loveland, CO, USA). The serology test results must have indicated at least one positive result to a mite (of any type) and at least one positive result to a pollen (of any type). A "positive" result was defined based on the laboratory's defined positive threshold. Apart from the above, sera were unselected as to age, breed, sex, number of positive serological reactions or any otherwise potentially qualitative or subjective factor. All samples in the serum bank that met the study criteria were included for analysis.

Sera were tested on a commercially available multiplex enzyme-labelled allergen-specific IgE assay (Polycheck Canis 20, Biocheck GmbH; Muenster, Germany). The format of the test is a cassette containing a membrane strip printed with bands of allergen extracts, each band corresponding to one allergen (Figure 1, red box). In addition, one band is printed with a MUXF3-rich extract (a carbohydrate chain purified from glycoprotein extracted from pineapple, *Ananas comosus*), which binds anti-CCD antibodies (Figure 1, white arrow). The strips were incubated sequentially with dog serum, biotinylated monoclonal anti-canine IgE (previously demonstrated as specific for canine IgE)⁷, avidin-alkaline phosphatase and then substrate. The resulting processed strips were dried and scanned on a flatbed scanner. Image analysis software (Biocheck GmbH) detected the densities of each band, along with densities of a series of control bands of graded density (Figure 1, blue box) and expressed the results in arbitrary units/mL (U/mL).

Results

Sera from 38 atopic canine patients met the pre-determined criteria outlined above. Patient age at the time of collection ranged from 1 to 12 years; date of sample collection ranged from November 2013 to April 2017. In total, nine of 38 sera (24%) had detectable anti-CCD IgE on the Polycheck assay. Although the colour intensity of the CCD indicator band varied (Figure 1), if positive, it was usually grossly visible and strong. Additionally, the nine dogs that were positive for anti-CCD IgE had an overall greater number (and greater median number) of positive reactions on

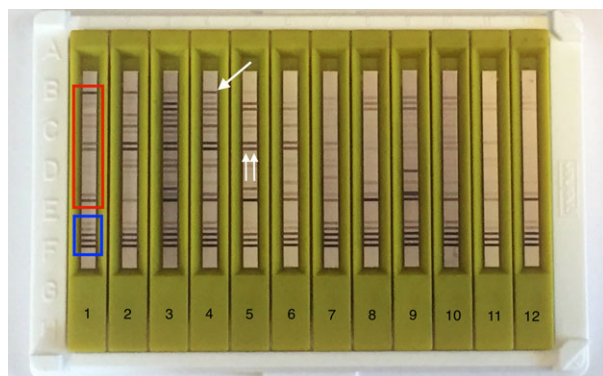


Figure 1. Polycheck Canis 20 Assay.

Each green cassette contains a strip with results from a single dog's serum, with individual allergens printed as bars across the strip. The single white arrow points to the bar delineating CCD positivity (variable magnitude of positivity evident between samples 1–6). Samples 1–6 are positive for anti-CCD IgE (however, bars are so faint in Sample 6 that the positive is undetectable without image scanning) and 7–12 are negative. The lower blue box outlines the standards; the red box outlines 20 individual allergen tests. The double white arrow indicates the position of grass pollen bars, which were always positive when anti-CCD IgE was detected.

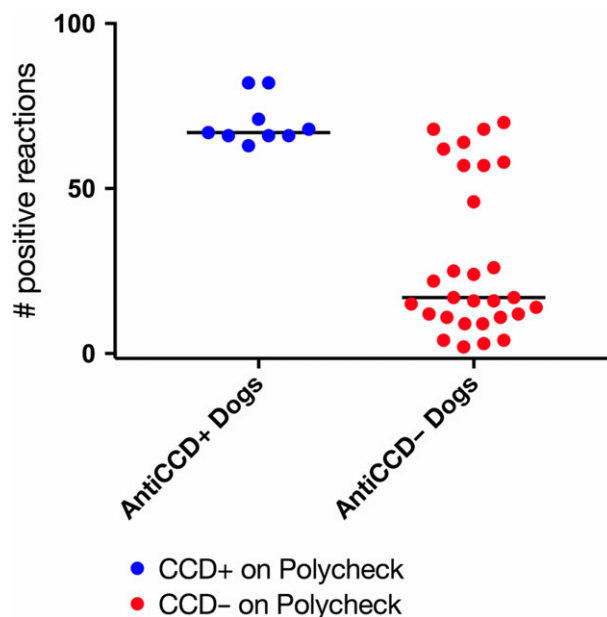


Figure 2. HESKA Allercept panel results in anti-CCD positive and negative dogs.

Each dot represents one dog. For dogs with anti-CCD IgE, the median number of positive reactions on HESKA allergen-specific IgE analysis was greater than the median for dogs without anti-CCD IgE.

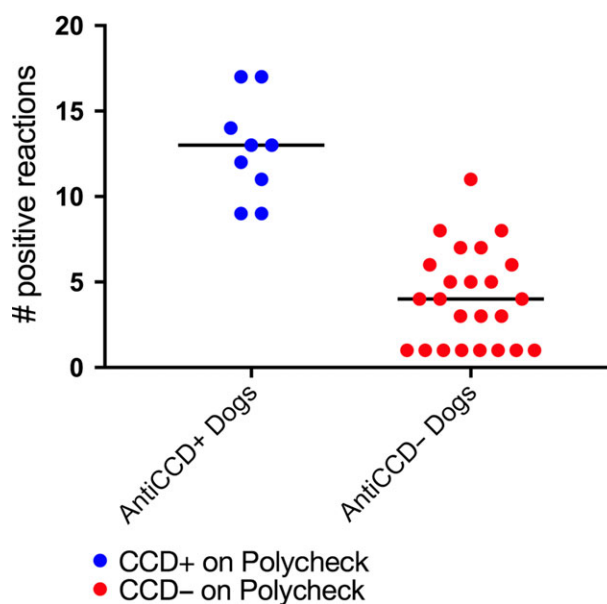


Figure 3. Polycheck Canis 20 panel results in anti-CCD positive and negative dogs.

Each dot represents one dog. For dogs with anti-CCD IgE, the median number of positive reactions on Polycheck analysis was greater than the median for dogs without anti-CCD IgE.

both Heska and Polycheck serum allergen tests (Figures 2 and 3). On the Polycheck assay for each sample, the magnitude of a given positive reaction was expressed via concentration (U/mL). Both the individual median strengths of positive reactions, as well as the total median strength of positive reactions (by group) were greater for dogs with anti-CCD IgE (Figure 4).

Nine of nine dogs with anti-CCD IgE sera were positive to both grass allergens evaluated on the Polycheck panel (rye pollen and grass mix), whereas only four of 29 anti-

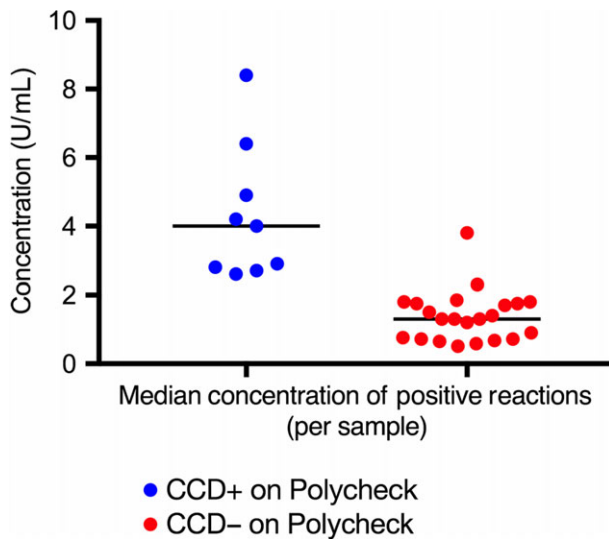


Figure 4. Quantitative results in anti-CCD positive and negative dogs.

Each dot represents one dog. For dogs with anti-CCD IgE, the median concentration (U/mL) of all positive reactions on the Polycheck assay was greater than that for anti-CCD IgE negative dogs.

CCD negative dogs were positive to both (Figure 1, double white arrow).

Discussion

Anti-CCD IgE is present in 20 to 70% of pollen-sensitized people.^{2,3,8} In the present study, we determined that anti-CCD IgE was detectable via a commercially available enzyme-labelled assay in nine of 38 (24%) pollen- and mite-sensitized dogs; this frequency mirrored that found in comparative human populations.

Studies in people have shown that the majority of CCDs are clinically irrelevant, even when they are strongly antigenic (i.e. capable of eliciting an immunological response). Mechanistically, this is largely attributed to their monovalency; a single IgE readily binds the single CCD epitope, yet no cross-linking (and thus, no mast-cell degranulation) will occur without at least two IgE binding sites/epitopes.⁹ In people, the clinical significance of anti-CCD IgE lies not in its ability to cause clinical signs of allergy, but in its ability to confound the results of allergen-specific IgE tests by creating clinically irrelevant or “false positive” test results.

The first and most widely cited study to document the confounding role of anti-CCD IgE in serum diagnostic tests was published in 2002.² This large cohort study was the first to document that CCD prevalence is increased among atopic individuals and further increased among polysensitized individuals, and, most importantly, that positivity to CCD on serum testing could not be replicated on skin prick testing. Subsequent studies have explored in detail the potential influence of various epidemiological factors on anti-CCD antibody prevalence within and

between populations, as well as how different types of CCD might be variably present or inhibited by certain compounds.^{5,8} One area of clinical importance is the recent advent of CCD “blockers” that can be used to competitively remove anti-CCD IgE from patient sera, thus decreasing “false positive” serology results.^{1,3}

Our results support an overall prevalence of anti-CCD IgE similar to that reported in people, but our sample size is small and the study population geographically limited. For example, the frequency of anti-CCD IgE in people can be as low as 4.7% if the allergic population is highly mite-sensitized and poorly pollen-sensitized (mites have few to no CCDs).⁸ Thus, further studies with larger and more regionally diverse populations would be strongly indicated to better characterize the frequency of anti-CCD IgE in the atopic canine population. Additionally, as in people, it would be important to compare frequency between sensitized and nonsensitized (i.e. nonatopic) dogs. The most important limitation of the present study is that the mere presence of anti-CCD may or may not be biologically relevant. Biological relevance could be established, for example, by comparison of serology test results with intradermal test results, or with *in vitro* lymphocyte stimulation with allergen. Such studies would potentially provide additional support for the confounding role that CCD plays specifically in serum allergen diagnostic tests, as well as help to identify specific populations more or less prone to possessing anti-CCD IgE.

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Résumé

Contexte – Les CCD (Cross-reactive carbohydrate determinants) sont définis comme des portions de carbohydrates des glycoprotéines des molécules fréquentes de la surface cellulaire de nombreuses plantes et espèces d’insectes. Les espèces de mammifères reconnaissent les CDD comme antigènes étrangers et peuvent développer une réponse immune humorale. Approximativement 20-37% des gens allergiques aux

graminées et au venin possèdent des IgE circulant anti-CDD ; ces anticorps sont généralement considérés cliniquement non pertinents. L' IgE anti-CDD est cependant reconnue comme une cause de faux positifs, cliniquement non correspondant aux tests allergiques sériques chez l'homme ; ce phénomène n'a pas été étudié chez l'animal.

Objectif – Déterminer si les IgE anti-CDD peuvent être détectés dans les séra des chiens atopiques de propriétaires et à quelle fréquence.

Sujets – Les séra de 38 chiens avec un diagnostic clinique de dermatite atopique et une sérologie antérieure mettant en évidence des anticorps IgE, définis comme résultat positif à au moins un acarien ou pollen (de toute sorte).

Méthodes – Les séra ont été analysés pour les IgE anti-CDD et les allergènes environnementaux avec un test spécifique d'allergènes disponible dans le commerce.

Résultats – Les IgE anti-CDD ont été détectés pour neuf des 38 (24%) séra des chiens atopiques. De même qu'avec leurs équivalents humains, tous les chiens avec des IgE anti-CDD avaient une forte réactivité sérologique aux pollens de graminées.

Conclusions et importance clinique – Les IgE anti-CDD peuvent fausser les tests sériques d'allergènes chez l'homme, ce qui pourrait être également vrai chez le chien. Des études supplémentaires sont nécessaires pour explorer les implications cliniques des IgE anti-CDD chez le chien, y compris le potentiel de ces IgE pour modifier les tests sériques d'IgE spécifiques d'allergènes utilisés pour le diagnostic clinique et s'ils sont pertinents pour la maladie clinique.

Resumen

Introducción – Los determinantes de carbohidratos con reacción cruzada (CCD) son porciones definidas de carbohidratos de las moléculas superficiales de glicoproteínas comunes a muchas especies de plantas e insectos. Las especies de mamíferos reconocen CCD como antígenos extraños y pueden montar respuestas inmunitarias humorales frente a ellos. Aproximadamente entre un 20-37% de personas alérgicas a pasto y veneno poseen IgE circulante contra CCD; estos anticuerpos generalmente se consideran clínicamente irrelevantes. La IgE anti-CCD es, sin embargo, reconocida como una causa de falsos positivos en resultados de pruebas de alérgenos séricos y clínicamente incongruentes en personas; este fenómeno no ha sido investigado en animales.

Objetivo – determinar si se puede detectar IgE anti-CCD en sueros de perros atópicos de propietarios particulares y con qué frecuencia se encuentran.

Animales – Sueros de 38 perros con un diagnóstico clínico de dermatitis atópica y evidencia serológica previa de anticuerpos IgE, definidos como un resultado positivo para al menos un ácaro y polen (de cualquier tipo).

Métodos – Los sueros se analizaron para detectar IgE frente a CCD y alérgenos ambientales con un ensayo múltiple de IgE específica de alérgeno marcado con enzimas y comercialmente disponible.

Resultados – Se detectó IgE anti-CCD en nueve de 38 (24%) de los sueros atópicos caninos. Al igual que en casos de seres humanos, todos los perros con IgE anti-CCD tenían una fuerte reactividad serológica al polen de hierba.

Conclusiones e importancia clínica – la IgE anti-CCD puede confundir las pruebas de alérgenos serológicos en personas; lo mismo podría ser cierto en perros. Se necesitan más estudios para investigar las implicaciones clínicas de la IgE anti-CCD en perros, incluida la posibilidad de que estos anticuerpos afecten las pruebas de IgE específicas de alérgenos en suero utilizadas para el diagnóstico clínico y si son relevantes para la enfermedad clínica.

Zusammenfassung

Hintergrund – Kreuzreaktive Kohlenhydratdeterminanten (CCD) werden als Kohlenhydratanteile von Oberflächenmolekülen von Glykoproteinen definiert, die bei vielen Pflanzen und Insektenarten vorkommen. Säugetierspezies erkennen CCD als fremde Antigene und können ihnen gegenüber eine humorale Immunantwort entwickeln. Annähernd 20-37% von Gras und Gift allergischen Menschen besitzen zirkulierende IgE gegen CCD; diese Antikörper werden generell als irrelevant betrachtet. Anti-CCD IgE kommt jedoch als Ursache für falsch positive, klinisch unschlüssige Serumallergietests beim Menschen vor; dieses Phänomen wurde bei Tieren bisher nicht untersucht.

Ziel – Festzustellen ob anti-CCD IgE in Sera von atopischen Hunden in Privatbesitz gefunden werden konnte und wie häufig das der Fall war.

Tiere – Sera von 38 Hunden mit der Diagnose einer klinischen atopischen Dermatitis und vorhergehendem serologischen Auftreten von IgE Antikörpern, was als positives Ergebnis für zumindest eine Milbe und Pollen (verschiedene Typen) definiert wurde.

Methoden – Sera wurden auf IgE gegen CCD und Umweltallergene mittels kommerziell erhältlichem Multiplex Enzyme-labelled Allergen-spezifischem IgE Assay analysiert.

Ergebnisse – Anti-CCD IgE wurden in neun von 38 (24%) der atopischen Hundesera gefunden. So wie bei den vergleichbaren Menschen zeigten alle Hunde mit anti-CCD IgE eine starke serologische Reaktivität auf Graspollen.

Schlussfolgerungen und klinische Bedeutung – Anti-CCD IgE können bei serologische Allergietests des Menschen interferieren; dasselbe könnte beim Hund der Fall sein. Weitere Studien sind nötig, um die klinischen Implikationen von anti-CCD IgE bei Hunden zu untersuchen. Dazu zählt auch die Beantwortung der Frage, ob diese Antikörper Allergen-spezifische Serum IgE Assays, die zur klinischen Diagnose verwendet werden beeinflussen und ob sie für die klinische Erkrankung eine Bedeutung haben.

要約

背景 – 交差反応性糖タンパク質糖鎖(CCD)とは、多くの植物や昆虫種に共通した糖タンパク質細胞表面分子の炭水化物部位である。哺乳動物種はCCDを外来抗原として認識し、体液性免疫応答によって対応させる。草および毒性アレルギー患者の約20~37%がCCDに対する末梢血IgEを有する。これらの抗体は臨床的には重要でないと考えられている。一方でヒトでは抗CCD IgEが、血清アレルギー試験で臨床と一致しない偽陽性の結果を引き起こす原因として認識されているが、この現象は動物では調べられていない。

目的

本研究の目的は、飼育されているアトピー犬の血清中に抗CCD IgEが検出されるかどうか、またどれくらいの頻度で検出されるかを決定することである。

被験動物

アトピー性皮膚炎の臨床診断に合致し、少なくとも1つのダニおよび花粉(いずれのタイプのもの)に対して陽性を示すことでIgE抗体の血清学的証拠を有すると定義された38匹の犬由来血清

方法

市販のマルチプレックス酵素標識アレルギー特異的IgEアッセイを用いて、CCDおよび環境アレルギーに対する血清IgEを解析した。

結果 – 抗CCD IgEは、アトピー犬血清38例中9例(24%)で検出された。ヒトの抗CCD IgEと同様、抗CCD IgEを有する全てのイヌは、草花粉に対する強い血清学的反応を示した。

結論と臨床的重要性 – 抗CCD IgEはヒトの血清学的アレルギー検査に混乱をもたらすが、同様の現象が犬にも当てはまるかもしれない。これらの抗体が臨床診断に用いられる血清アレルギー特異的IgEアッセイに影響を及ぼす可能性や、それらが臨床的疾患に関連しているかどうかなど、イヌにおける抗CCD IgEの臨床的意義を調査するためのさらなる研究が必要である。

摘要

背景 – 许多植物和昆虫共有的糖蛋白细胞表面分子,其碳水化合物部分被定义为交叉碳水化合物决定物(CCD)。哺乳动物将CCD作为外来抗原,并能引发轻微的免疫应答。大约20-37%对草和昆虫毒液过敏的人,有抗CCD循环IgE;这些抗体通常与临床症状无关。然而,在人类,认为通过血清过敏原检测抗CCD IgE,易造成与临床症状不相符的假阳性结果,这种现象在动物还未被研究。

目的 – 确定私家异位性皮炎患犬血清中是否存在抗CCD IgE,及其检测率。

动物

临床诊断为异位性皮炎的38只犬,先前曾确认血清存在IgE抗体,本次血清学检测至少一种螨虫或花粉(任意类型)为阳性结果。

方法

通过市售商业化多重酶标抗原特异性IgE试验,检测血清中的抗CCDIgE和环境过敏原。

结果 – 38只异位性皮炎患犬中有9只(24%)血清检测到抗CCDIgE。与人类一样,所有携带抗CCDIgE的犬,对植物花粉有更强的血清学反应。

结论和临床意义 – 人的抗CCDIgE造成血清过敏原检测结果混乱;对于犬也是如此。需要更多研究调查犬抗CCDIgE的临床意义,包括在临床诊断中,这些抗体对血清抗原特异性IgE试验的影响,以及是否与临床疾病相关。

Resumo

Contexto – Os determinantes de carboidratos de reação cruzada (CCD, do inglês, “cross-reactive carbohydrate determinants”) são fragmentos de carboidratos presentes em moléculas de glicoproteínas de localizadas nas superfícies celulares comuns a diversas espécies de plantas e insetos. Os mamíferos podem reconhecer CCD como antígenos não próprios e montar uma resposta imune humoral contra os mesmos. Aproximadamente 20-37% de pessoas alérgicas a gramíneas e venenos possuem IgE contra CCD circulantes; estes anticorpos geralmente são considerados irrelevantes. Entretanto, sabe-se que, em humanos, IgE anti-CCD podem causar reações falso-positivas e resultados clinicamente incongruentes em testes alérgicos sorológicos. Este fenômeno ainda não foi investigado em animais.

Objetivo – Determinar se IgE anti-CCD podem ser detectados no soro de cães atópicos de clientes e a frequência em são encontrados.

Animais – Soro de 38 cães clinicamente diagnosticados com dermatite atópica que apresentaram anteriormente respostas positivas à presença de anticorpos IgE específicos para ao menos um ácaro e pólen (de qualquer tipo).

Métodos – Realizou-se uma análise sérica da presença de IgE contra CCD e alérgenos ambientais utilizando um ensaio imunoenzimático multiplex para IgE alérgeno-específico comercial.

Resultados – Detectou-se IgE anti-CCD no soro de nove dos 38 (24%) cães atópicos. Assim como nos humanos, todos os cães com IgE anti-CCD apresentaram reações séricas fortes a pólenes de gramíneas.

Conclusões e importância clínica – Anticorpos IgE anti-CCD podem confundir os resultados de testes alérgicos sorológicos em humanos, e é provável que o mesmo ocorra em cães. Mais estudos são necessários para investigar as implicações clínicas de IgE anti-CCD em cães, incluindo o potencial destes anticorpos de causarem alterações nos ensaios de IgE alérgenos-específicos utilizados na rotina, e a sua relevância nas manifestações clínicas da doença.