

A pilot study of total and allergen-specific IgE serum levels during anestrus, estrus and pregnancy in healthy female dogs

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Background – Allergen-specific IgE serology is used for the determination of sensitization status in dogs with atopic dermatitis; the influence of the female reproductive cycle on the results of such methods has not been studied in dogs.

Objectives – To compare the total and allergen-specific IgE of healthy bitches during anestrus, estrus and pregnancy.

Animals – Eight privately owned, healthy bitches.

Methods – Total and allergen-specific IgE levels were determined in eight bitches at three different time-points of their reproductive cycle: anestrus, estrus and pregnancy.

Results – Total IgE was significantly decreased (median: 74%) in female dogs during pregnancy when compared to anestrus. In 14 of 216 (6%), allergen-specific IgE test results were variably positive and negative at different stages of the reproductive cycle. This variation, however, was not related to changes in total serum IgE levels.

Conclusions – Total IgE serum levels are reduced during pregnancy in female dogs. However, results of one allergen-specific IgE test did not appear to be markedly altered by the reproductive cycle in healthy bitches.

Introduction

Atopic dermatitis (AD) is a common allergic skin disease of dogs and IgE serology is used to identify potentially clinically relevant environmental allergens. It has been postulated that female steroid hormones may influence the incidence of allergic diseases in women.¹ Conflicting results have been reported for progesterone and estradiol levels, and for total and allergen-specific IgE, and their relationship to menstruation and pregnancy.^{1–4} To the best of the authors' knowledge, there is no information on the relationship between the reproductive cycle and IgE in dogs. Our objectives were to examine the relationship between total IgE, allergen-specific IgE and the reproductive status of healthy female dogs.

Materials and methods

Eight privately owned, physically healthy breeding bitches were included and sera were collected on three occasions: during anestrus (approximately four weeks pre-estrus), estrus and mid-term gestation. The phases of the reproductive cycle were confirmed based on the bitch's behaviour, vaginal cytology, progesterone levels and abdominal ultrasound. All bitches were regularly dewormed (every third month with a preparation containing praziquantel, fenbendazole and pyrantel) and treated for ectoparasite infestations (all but one (Dog 4) were treated with oral fluralaner or spot-on preparation moxidectin/imidacloprid); none exhibited any signs of allergic disease. The environment of the dogs remained unchanged during the study. All dogs lived in a household in central Europe, a geographical area where pollen seasons are short and with a low incidence of pollen allergy in dogs.

Samples and analyses

Blood was drawn by venipuncture then allowed to clot at room temperature, and centrifuged. All sera were stored at –80°C until shipment. Total IgE was measured using a commercial canine IgE ELISA quantitation kit (Bethyl Laboratories; Montgomery, AL, USA) and results were expressed in ng/mL. All samples were analysed in duplicate using a previously reported assay.⁵ Another study permitted the determination of the range of total IgE levels in normal dogs.⁶

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Conflicts of interest: No conflicts of interest have been declared.

Canine allergen-specific IgE serum levels were determined by an external private laboratory [Sensitest, Avacta Animal Health; Wetherby, Leeds, UK (analyses run by Gallileo; Vandoeuvre-les-Nancy, France)]. All samples were analysed at the same time. Coefficients of variation for this assay were 4% (intra-assay) and 15% (inter-assay); results were expressed in arbitrary units calculated based on the optic densities and in reaction classes ranging from 0 (negative) to 4 (strongly positive).⁷ Allergens are listed in Table S1.

Statistical analyses

As total IgE serum levels were not normally distributed, the Wilcoxon matched pairs signed rank test was used to compare levels in anestrus, estrus and pregnancy. Serum levels of IgE specific for nine different nonseasonal allergens were assessed for each dog and at the three reproductive cycle time points (216 tests). For each dog, results were considered different when at least one result was positive and one result negative for the same allergen (reaction classes). Additionally, the number of positive tests for each dog at each time-point= was calculated. The correlation between this number and total IgE levels was evaluated using Spearman's rank correlation test. Finally, the number of positive results in one specific reproductive cycle stage which became negative in subsequent stages were collated. Statistical analyses were carried out with Graphpad Prism 7 (Graphpad software, La Jolla, CA, USA).

Results

Total IgE

Total IgE serum levels are reported in Table 1. Total IgE serum levels were lower in six of eight bitches (75%) during estrus and in seven of eight bitches (88%) during pregnancy compared to those during anestrus; IgE levels were lower in six of eight bitches (75%) during pregnancy than during estrus. Total IgE in Dog 4 (not treated for ectoparasites) did not show major variations. The difference between total IgE serum levels during anestrus and pregnancy was statistically significant ($P = 0.02$), whereas other comparisons, between the combined results for all eight dogs for each stage of the reproductive cycle, were not. Interestingly, the changes in total IgE levels between different stages of reproduction were highly variable between bitches (Table 1).

Allergen-specific IgE

The number of allergens with positive test results in the different reproductive stages is presented in Table 2. There were differences in positive results for allergen-specific IgE in subsequent phases of the reproductive

cycle (becoming positive or negative) in 14 of 216 (6%) tested allergens (Table 3). Whenever observed, such changes in reactivity only affected levels of IgE specific for moulds and forage mite *Acarus siro*, as the house dust-mite specific IgE seropositive and seronegative results did not vary between reproductive cycles (Table S1). The dog that was not treated for ectoparasites did not have major (positive versus negative) variations. There was no significant correlation between total IgE levels and the number of allergen-specific positive tests (Spearman's correlation test, $r = 0.34$; $P = 0.11$).

Discussion

To the best of the authors' knowledge, this is the first study assessing the potential relationship between the reproductive cycle and serum IgE levels in female dogs. As our study included only a small number of dogs, the results should be interpreted with caution. Another

Table 2. Number of nonseasonal allergens with positive specific IgE during different stages of reproduction in eight bitches

	Anestrus	Estrus	Pregnancy
Dog 1	3	4	3
Dog 2	6	4	4
Dog 3	4	4	6
Dog 4	3	3	3
Dog 5	3	2	2
Dog 6	9	9	9
Dog 7	4	4	4
Dog 8	2	3	3
Total	34	33	34

Table 3. Changes of positive status in allergen-specific IgE serology during different stages of the reproduction cycle in bitches

Reproductive stage/number of positive results	Number of subsequent negative results		
	Anestrus	Estrus	Pregnancy
Anestrus/34	-	3	3
Estrus/33	2	-	1
Pregnancy/34	3	2	-

Table 1. Total IgE serum levels during different stages of the reproduction cycle in eight bitches.

Breed/age (years)	Anestrus	Estrus	Estrus versus anestrus %	Pregnancy	Pregnancy versus anestrus %	Pregnancy versus estrus %
English bulldog/4	107.4	15.5	-86	36.3	-66	135
Samoyed/5	432.5	537.2	24	27.4	-94	-95
Broholmer/7	280.3	137.5	-51	8.9	-97	-94
Samoyed/7	61.2	683	12	31.8	-48	-53
Scottish shepherd dog/6	62.1	13.0	-79	12.2	-80	-6
Boston terrier/3	67.0	62.6	-7	55.4	-17	-12
Australian shepherd dog/3	446.8	134.7	-70	56.4	-87	-58
Hungarian greyhound/7	36.3	35.5	-2	53.2	47	50
Median	87.2	65.5	-29	34.1	-73	-33
Minimum	36.3	13.0	-86	8.9	-97	-95
Maximum	446.8	537.2	24	56.4	47	135

The difference between total IgE serum levels during anestrus and pregnancy was statistically significant ($P = 0.02$), whereas other comparisons, between the combined results for all eight dogs for each stage of the reproductive cycle, were not.

limitation is the lack of a control group of neutered dogs without the possible influence of a reproductive cycle.

One should also keep in mind that several factors such as gender, neutering, breed, season and environment may influence total IgE in dogs.^{6,8} Atopy is another cause that may lead to changes in total IgE levels, although our study subjects did not show any clinical signs of AD and some studies have failed to demonstrate increased IgE levels in allergic dogs compared to healthy normal dogs.⁵

It is worth mentioning that allergen-specific IgE levels were very high in some dogs (especially Dog 6), although none of these dogs were considered to be clinically allergic. Additionally, almost all dogs were positive for house dust mites. The inability of allergen testing to differentiate between atopic and nonatopic dogs is well known, and has been documented previously.^{9,10} In contrast to the total IgE serum levels, values for allergen-specific IgE, for nonseasonal allergens, did not change markedly in different stages of the reproductive cycle in bitches.

In summary, total serum IgE concentrations were reduced during pregnancy in a small group of healthy dogs. Pregnancy did not have an influence on serum allergen-specific IgE. Further studies are indicated in dogs (bitches) with atopic disease, investigating the relationship of total and allergen-specific IgE with hormonal status.

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Supporting Information

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

Table S1. Allergen-specific IgE test results, for selected nonseasonal allergens, for eight dogs during the reproductive cycle.

Résumé

Contexte – La sérologie d'IgE spécifiques d'allergènes est utilisée pour déterminer la sensibilisation des chiens atteints de dermatite atopique ; l'influence du cycle reproducteur chez la femelle sur les résultats n'a pas été étudiée chez le chien.

Objectifs – Comparer les IgE totaux et spécifiques d'allergènes chez les femelles saines au cours de l'anoestrus, l'oestrus et la gestation.

Sujets – Huit chiennes saines de propriétaires privés.

Méthodes – Les taux d'IgE totaux et spécifiques d'allergènes ont été dosés chez huit chiennes à trois moments différents de leur cycle : anoestrus, oestrus et gestation.

Résultats – Les IgE totaux étaient significativement plus bas (médiane : 74%) chez les femelles au cours de la gestation comparé avec l'anoestrus. Pour 14 des 216 (6%) tests d'IgE spécifiques d'allergènes, les résultats étaient variablement positifs ou négatifs selon les moments du cycle de reproduction. Cette variation n'a pas été reliée aux changements des taux sériques d'IgE totaux.

Conclusions – Les taux sériques d'IgE totaux sont plus bas en cours de gestation chez les chiennes. Cependant, les résultats des tests d'IgE spécifiques d'allergène ne semblent pas modifiés par le cycle reproducteur chez les chiennes saines.

Resumen

Introducción – la serología de IgE específica de alérgeno se usa para determinar el estado de sensibilización en perros con dermatitis atópica; la influencia del ciclo reproductivo femenino en los resultados de tales métodos no se ha estudiado en perros.

Objetivos – comparar la IgE total y la específica de alérgeno de perras sanas durante anestro, estro y embarazo.

Animales – Ocho perras sanas de propietarios particulares.

Métodos – Se determinaron los niveles de IgE total y de específicas de alérgenos en ocho perras en tres momentos diferentes de su ciclo reproductivo: anestro, estrógeno y embarazo.

Resultados – IgE total disminuyó significativamente (mediana: 74%) en perros hembra durante el embarazo en comparación con el anestro. En 14 de 216 (6%), los resultados de las pruebas de IgE específicas

para alérgenos fueron positivos y negativos de forma variable en diferentes etapas del ciclo reproductivo. Sin embargo, esta variación no se relacionó con cambios en los niveles séricos de IgE total.

Conclusiones – los niveles séricos de IgE total se redujeron durante el embarazo en perros hembras. Sin embargo, los resultados de una prueba de IgE específica de alérgeno no parecen estar marcadamente alterados por el ciclo reproductivo en perras sanas.

Zusammenfassung

Hintergrund – Die Allergen-spezifische IgE Serologie wird zur Bestimmung des Sensibilisierungsstatus bei Hunden mit atopischer Dermatitis verwendet; der Einfluss des weiblichen Reproduktionszyklus auf die Ergebnisse einer solchen Methode ist bei Hunden bisher nicht untersucht worden.

Ziele – Ein Vergleich des Gesamt- und Allergen-spezifischen IgE von gesunden Hündinnen im Anöstrus, Östrus und während der Trächtigkeit.

Tiere – Acht gesunde Hündinnen in Privatbesitz.

Methoden – Gesamt- und Allergen-spezifische IgE Werte wurden bei acht Hündinnen zu drei unterschiedlichen Zeitpunkten ihres Reproduktionszyklus bestimmt: im Anöstrus, im Östrus und während der Trächtigkeit.

Ergebnisse – Gesamt IgE war bei den Hündinnen während der Trächtigkeit im Vergleich zum Anöstrus signifikant erniedrigt (Median: 74%). Bei 14 von 216 (6%) waren die Allergen-spezifischen IgE Test Ergebnisse zu verschiedenen Zeitpunkten des Reproduktionszyklus unterschiedlich positiv und negativ. Diese Variation stand jedoch nicht im Zusammenhang mit den Gesamt IgE Werten.

Schlussfolgerungen – Gesamt IgE Werte sind während der Trächtigkeit von weiblichen Hunden reduziert. Die Ergebnisse eines Allergen-spezifischen IgE Tests schienen durch den Reproduktionszyklus bei gesunden Hündinnen nicht deutlich verändert zu sein.

要約

背景 – アレルゲン特異的IgE血清学的解析は、アトピー性皮膚炎の犬におけるアレルゲン感作を確認するために用いられる。しかし、本法の結果に雌犬の生殖周期が影響するかどうかは、現在のところ報告されていない。

目的 – 本研究の目的は、発情休止期、発情期および妊娠期間中の健康な雌犬における総IgE値およびアレルゲン特異IgE抗体価を比較することである。

被験動物 – 飼育された健康な雌犬8頭。

方法 – 生殖周期の3つの異なる時点(発情休止期、発情期および妊娠期間)における総IgE値及びアレルゲン特異的IgE抗体価を8頭の雌犬で測定した。

結果 – 総IgE値は、発情休止期よりも妊娠期間中の雌犬において有意に低値を示した(低下の中央値:74%)。216サンプル中14サンプル(6%)において、アレルゲン特異的IgE抗体価が、生殖周期の異なる段階により陽性または陰性となった。しかし、この変化は総血清IgE値の変化とは関連していなかった。

結論 – 妊娠期間中の雌犬では総IgE血清値の低下が認められた。しかしながら、アレルゲン特異的IgE抗体価は、健康な雌犬の生殖周期による明らかな変化を認めなかった。

摘要

背景 – 抗原特异性IgE血清学检测,用于测定犬异位性皮炎的致敏状态;尚无关于雌性犬生殖周期影响这些方法所得结果的研究。

目的 – 比较健康母犬乏情期、发情期和怀孕期的总IgE和抗原特异性IgE。

动物 – 八只私家、健康母犬。

动物 – 在八只母犬生殖周期的三个不同的时间点:乏情期、发情期和怀孕期,测定了其总IgE和抗原特异性IgE水平。

结果 – 与乏情期相比,怀孕期间母犬的总IgE水平显著降低(中值:74%)。216例中的14例(6%),在生殖周期的不同阶段,抗原特异性IgE检测结果阳性和阴性变化不定。然而,这种变化与血清总IgE水平的变化无关。

结论 – 在怀孕期间,母犬的血清总IgE水平降低。然而,在健康母犬的生殖周期,单项抗原特异性IgE检测结果无明显改变。

Resumo

Contexto – O teste sorológico de IgE alérgeno-específica é utilizado para a determinação do status de sensibilização em cães com dermatite atópica; a influência do ciclo reproductivo feminino nos resultados destes métodos ainda não foi estudada em cães.

Objetivos – Comparar os níveis séricos de IgE total e IgE alérgeno-específica em cadelas saudáveis durante o anestro, estro e prenhez.

Animais – Oito cadelas saudáveis de clientes.

Métodos – Os níveis séricos de IgE total e IgE alérgeno-específica foram determinados em oito cadelas, em três períodos distintos dos seus ciclos reproductivos: anestro, estro e prenhez.

Resultados – A IgE total estava significativamente reduzida (mediana: 74%) nas cadelas durante a prenhez quando comparado ao anestro. Em 14 de 216 (6%), os resultados da dosagem de IgE alérgeno-específica

foram variavelmente positivos e negativos nos diferentes estágios do ciclo reprodutivo. Entretanto, estas variações não foram relacionadas às alterações nos níveis séricos de IgE total.

Conclusões – Há redução nos níveis séricos de IgE total durante a prenhez em cadelas. Entretanto, os resultados de um teste de dosagem sérica de IgE alérgeno-específica aparentemente não sofreram alterações marcantes devido às distintas fases do ciclo reprodutivo.

Supporting Information

Table S1. Allergen-specific IgE test results, for selected non-seasonal allergens, for eight dogs during the reproductive cycle

Dog	sexual cycle	Acarus	Lepidog.	Df	Dp	Cat	Human	Penicillium	Aspergillus	Alternaria	Total positive
Dog1	Anoestrus	4	0	5	2	0	0	0	0	0	3
Dog1	Oestrus	2	0	3	3	4	0	0	0	0	4
Dog1	Pregnancy	2	0	3	3	0	0	0	0	0	3
Dog2	Anoestrus	5	2	5	5	0	0	2	1	0	6
Dog2	Oestrus	5	2	5	5	0	0	0	0	0	4
Dog2	Pregnancy	2	1	4	2	0	0	0	0	0	4
Dog3	Anoestrus	5	4	5	4	0	0	0	0	0	4
Dog3	Oestrus	5	1	5	4	0	0	0	0	0	4
Dog3	Pregnancy	1	1	3	3	0	0	3	0	2	6
Dog4	Anoestrus	2	0	3	2	0	0	0	0	0	3
Dog4	Oestrus	2	0	3	3	0	0	0	0	0	3
Dog4	Pregnancy	2	0	3	2	0	0	0	0	0	3
Dog5	Anoestrus	4	0	5	3	0	0	0	0	0	3
Dog5	Oestrus	0	0	2	1	0	0	0	0	0	2
Dog5	Pregnancy	0	0	2	2	0	0	0	0	0	2
Dog6	Anoestrus	4	3	4	4	2	2	2	2	2	9
Dog6	Oestrus	4	3	5	4	2	2	2	2	2	9
Dog6	Pregnancy	4	3	5	4	3	3	3	3	3	9
Dog7	Anoestrus	5	3	5	5	0	0	0	0	0	4
Dog7	Oestrus	5	4	5	4	0	0	0	0	0	4
Dog7	Pregnancy	5	1	5	4	0	0	0	0	0	4
Dog8	Anoestrus	0	0	2	1	0	0	0	0	0	2
Dog8	Oestrus	4	0	5	2	0	0	0	0	0	3
Dog8	Pregnancy	2	0	3	3	0	0	0	0	0	3

Each dog has data points for three parts of the sexual cycle - anoestrous, oestrous and pregnancy.

Results are expressed in arbitrary units and are calculated on the basis of optical densities and designated into reaction classes ranging from 0 (negative) to 4 (strongly positive). Test samples submitted to Sensitest, Avacta Animal Health, Wetherby, Leeds, United Kingdom; Analyses run by Gallileo, Vandoeuvre-les-Nancy, France).

Red cells are negative and positive results for the same dog.

Lepidog. – *Lepidoglyphus*; Df *Dermatophagoides farinae*; Dp *Dermatophagoides pteronyssinus*