

# **Epidemiology of Canine Atopic Dermatitis**

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## Abstract

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Canine atopic dermatitis (CAD) is a chronic, allergic skin disease associated with IgE-mediated reactions to environmental allergens. Atopic dermatitis/eczema in humans shares many similarities with CAD and is an increasing problem in industrialized countries. This increase has been attributed to lifestyle and environment factors. The current knowledge about the epidemiology of CAD is limited. The aim of this thesis was therefore to investigate the incidence of and potential risk factors for the development of CAD.

Three of the included studies involve the use of a large animal-insurance database. The database includes information about a large number of healthy and diseased individuals over time, but it was not collected for research purposes and data-quality issues needed to be addressed. A validation of the diagnosis CAD in the insurance database showed that although the vast majority of the recorded cases could be considered allergic, the important differential diagnosis cutaneous adverse food reactions had not been ruled out for many patients. The overall incidence rate of CAD was 1.7 cases per 1000 dog years at risk. Several factors were found to be associated with an increased risk of CAD in the insured population; living in an urban area or in the south of Sweden, being born in the autumn and belonging to a high-risk breed. Furthermore, a spatial analysis showed that the incidence of CAD increased by increasing human population density and increasing annual rainfall, and was decreased in the north of Sweden and if there was no veterinary dermatologist present in the county. Finally, a case-control study was performed where 12 veterinarians collected CAD cases from the three identified high-risk breeds; boxer, bullterrier and West Highland white terrier. The main finding was that feeding a diet containing home-made/ non-commercial ingredients to the bitch during lactation protected her offspring from developing CAD.

In conclusion, a strong breed predisposition for CAD was seen. Evidence of an increased incidence of CAD in densely populated areas exists but might be biased by the locations of veterinary dermatologists. The potential of using diet for primary prevention of CAD is interesting but randomized controlled clinical trials are required to support this finding.

*Keywords:* canine atopic dermatitis, incidence, risk factors, validation, insurance database, spatial analysis, case-control study

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*Prurio ergo sum*

# Contents

<b>Background</b>	<b>9</b>
Canine atopic dermatitis	9
<i>Clinical manifestations</i>	
<i>Diagnosis</i>	
<i>Occurrence</i>	
<i>Pre-disposing factors</i>	
<i>Prevention</i>	
<i>Clinical relevance</i>	
Atopic dermatitis in humans	12
<i>Pre-disposing factors</i>	
<i>Immunology and “The hygiene hypothesis”</i>	
Comparison between canine and human atopic dermatitis	14
Estimating disease frequency in companion animals	15
<i>Measures of disease frequency</i>	
<i>Sources of population data for companion animals</i>	
<b>Aims</b>	<b>18</b>
<b>Methodological considerations</b>	<b>19</b>
Study populations	19
<i>Insured population</i>	
<i>Case-control population</i>	
Validation of the diagnosis CAD among insured dogs	20
Incidence rate calculation	21
Risk factor analyses	21
<i>Cox regression with a random error term</i>	
<i>Spatial analysis</i>	
<i>Logistic regression with random effects</i>	
<b>Results and discussion</b>	<b>22</b>
The importance of eliminating adverse food reactions	22
CAD incidence varies by breed	23
Spatial patterns in the distribution of CAD cases	24
Early diet and its effect on CAD occurrence	27
Other pre-disposing factors	28
<b>Conclusions</b>	<b>29</b>
<b>Implications for future research</b>	<b>30</b>
<b>References</b>	<b>31</b>
<b>Acknowledgements</b>	<b>36</b>



## Appendix

The thesis is based on the following papers which will be referred to in the text by Roman numerals:

I. Nødtvedt A, Bergvall K, Emanuelson U, Egenvall A. (2006). Canine atopic dermatitis: validation of recorded diagnosis against practice records in 335 insured Swedish dogs. *Acta Veterinaria Scandinavica* 48 (8).

(Available online at: <http://www.actavetscand.com/content/48/1/8>)

II. Nødtvedt A, Egenvall A, Bergvall K, Hedhammar Å. (2006). Incidence of and risk factors for atopic dermatitis in a Swedish population of insured dogs. *Veterinary Record* 159, 241 - 246.

III. Nødtvedt A, Guitian J, Egenvall A, Emanuelson U, Pfeiffer DU. (2007). The spatial distribution of atopic dermatitis cases in a population of insured Swedish dogs. *Preventive Veterinary Medicine* 78, 210 - 222.

IV. Nødtvedt A, Bergvall K, Sallander M, Egenvall A, Emanuelson U, Hedhammar Å. A case-control study of risk factors for canine atopic dermatitis among boxers, bullterriers and West Highland white terriers in Sweden. (*Submitted manuscript.*)

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## Abbreviations

AD	Atopic dermatitis
CAD	Canine atopic dermatitis
CAFR	Cutaneous adverse food reactions
CI	Confidence interval
EB	Empirical Bayes
DYAR	Dog years at risk
HDM	House dust mite
HPD	Human population density
IR	Incidence rate
PAF	Population attributable fraction
PCA	Postal code area
T <sub>H</sub> 1	Type 1 helper T cells
T <sub>H</sub> 2	Type 2 helper T cells
WHWT	West Highland white terrier



# Background

## Canine atopic dermatitis

Canine atopic dermatitis (CAD) has been defined as “a genetically-predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features (...) associated most commonly with IgE antibodies to environmental allergens” (Olivry et al., 2001). In Sweden, as well as in many other European countries, the most frequently reported allergen reactions among dogs with CAD are towards house dust mites (HDM) (Öhlén, 1992; Saridomichelakis et al., 1999). Other groups of allergens in CAD-positive dogs include epithelia, pollens, moulds, dusts and feathers.

Atopic dermatitis was first reported in a dog 65 years ago, and received increasing attention in the later half of the 20<sup>th</sup> century (Griffin & DeBoer, 2001). Despite of this continuing interest, many issues remain unresolved regarding the clinical manifestations, diagnosis, occurrence and pre-disposing factors for CAD.

### *Clinical manifestations*

Pruritus is the main clinical manifestation of CAD, and it typically affects the face, ears, paws, extremities, axillae and/or ventrum (Griffin & DeBoer, 2001). The extent of primary lesions involved in CAD is somewhat unclear, although the presence of erythema appears to be generally accepted (Hillier & Olivry, 2004). Marsella et al. (2006) report that high-IgE responder beagles which had been epicutaneously sensitized to HDM developed papular eruptions in addition to pruritus and erythema following environmental allergen challenge. The severity of the lesions was dose-dependent and reportedly mimicked those in privately owned patients with CAD, and this was taken as an indication that these were primary lesions (Marsella et al., 2006). Reported secondary lesions of CAD include self-induced effects of licking and scratching such as salivary staining, excoriations and alopecia. Scaling, hyperpigmentation and dry lustreless hair-coat are other common findings. Furthermore, otitis externa and secondary bacterial infections are typical features of CAD patients and conjunctivitis has also been described in many cases. The typical time of onset for CAD is between six months and three years of age, and most affected dogs will eventually develop non-seasonal clinical signs even though the disease episodes can be seasonal initially (Griffin & DeBoer, 2001).

### *Diagnosis*

No definitive diagnostic test exists for CAD and the diagnosis is instead based upon the typical clinical presentation after exclusion of relevant differential diagnoses. A list of minor and major criteria for the diagnosis was proposed in the 1980s, inspired by similar lists used for the diagnosis of human atopic dermatitis (Willemse, 1986). The criteria proposed by Willemse (1986) have been widely used by veterinary dermatologists when diagnosing CAD, but have not been

formally evaluated in terms of sensitivity and specificity. It has been argued that neither these nor any other proposed checklists of clinical criteria are perfect, and that the only way of confirming a state of hypersensitivity is by provocative testing using offending allergens in affected patients (DeBoer & Hillier, 2001a). This is not feasible in a clinical setting, but it should be kept in mind that the diagnosis CAD needs to be based on careful evaluation of the patient and elimination of other causes of pruritus and not on a single feature or test.

An important point regarding the disease is that dogs can react to ingested food with clinical signs that are indistinguishable from those of CAD. Many dogs that react to food with pruritus and inflammation of the skin do not have IgE-mediated hypersensitivity towards ingested proteins, and the disease complex is termed cutaneous adverse food reactions (CAFR) due to an uncertainty regarding pathogenesis (Hillier & Griffin, 2001a). The diagnosis of CAFR is ruled out by performing a hypoallergenic diet trial followed by rechallenge, which needs to be done before a diagnosis of CAD can be established (Griffin & DeBoer, 2001). The exact relationship between CAD and CAFR is unclear, but several studies report that a fraction of dogs with CAD (3 – 13%) have concurrent CAFR (Hillier & Olivry, 2004).

Once the diagnosis of CAD has been established it is often of interest to determine which specific allergens a patient reacts towards. This is done by either serum-based (DeBoer & Hillier, 2001b) or intradermal allergy tests (Hillier & DeBoer, 2001). The aim of the allergen identification testing is to select allergens for immunotherapy or allergen avoidance, and it has been repeatedly emphasized that these tests are not useful for the initial clinical diagnosis of CAD because even clinically healthy animals can display positive reactions (DeBoer & Hillier, 2001a). More recently, Hillier and Olivry (2004) suggested that the diagnosis of CAD should be based on the following criteria:

1. Clinical features: pruritus and skin lesions affecting any of the following body sites: face, ears, ventral trunk (especially axillae and groin) and/or distal limbs.
2. Elimination of differential diagnoses: flea allergy dermatitis, CAFR, sarcoptic acariasis, other pruritic mite infestations, Staphylococcal dermatitis and Malassezia dermatitis.
3. Supplementation of allergy tests: positive reactions on intradermal testing or with IgE specific serology should be an aid in clinical management of cases, but not form the basis of the diagnosis.

These criteria might be more practical from a clinical perspective than the criteria proposed by Willemse (1986), but also lack documentation in terms of sensitivity, specificity and predictive ability.

### *Occurrence*

The incidence and prevalence of CAD are both unknown. A prevalence of about 3 to 15% of the general population has been suggested, but these estimates are not based on reliable epidemiological data (Hillier & Griffin, 2001b). Some studies have related the number of CAD cases to all dermatology complaints in various

kinds of veterinary practice, as this will give a general impression of how common the problem is. Hillier and Griffin (2001b) reviewed the literature and found that between 3.3% and 30% of all cases with skin disease were reported to have CAD, depending on the type of practice studied. Not surprisingly, the proportion of CAD positive dogs tended to be higher in dermatology referral clinics than in general practice.

Estimating the incidence and prevalence of CAD is complicated by the fact that mild cases of disease are likely to be treated symptomatically, without the owner (or veterinarian) being aware of the underlying disease. The same problem of misclassification might apply to patients with recurrent otitis externa or bacterial dermatitis as the main presenting complaint. Finally, the lack of uniformly defined diagnostic criteria for CAD makes reliable and reproducible studies of the disease difficult to perform (Hillier & Olivry, 2004).

### *Pre-disposing factors*

Gender predilections for CAD have been reported for both males and females, and whether a difference in disposition between genders exist remains unresolved (Griffin & DeBoer, 2001).

It is generally accepted that there is a genetic predisposition to CAD, because the disease is seen more commonly within certain breeds (Sousa & Marsella, 2001). The reported high-risk breeds vary between study populations and geographical regions. This might reflect genetic differences between regional dog populations but might also be an artefact because many studies do not take the distribution of breeds in the underlying population at risk into account. Sousa and Marsella (2001) compared reported high-risk breeds from ten different studies, and both the West Highland white terrier (WHWT) and boxer were reported to be at increased risk in several studies as were, among others, Labrador and golden retrievers.

In a British study, the heritability of CAD was estimated to be 0.47 in 32 litters of (mainly) Labrador and golden retrievers, suggesting that the genetic background contributed around 50% of the variability of disease occurrence (Shaw et al., 2004). It should be noted that these dogs were all from the “Guide dogs for the blind association”, and hence subject to fairly consistent management practices during their first two years of life. In a study of 33 litters of privately owned WHWTs, no clear pattern of heritability was detected but a tendency of CAD positive puppies to cluster within litters was observed (DeBoer & Hill, 1999). Even though genetic predisposition seems to be an important underlying feature for CAD, allergen exposure in combination with multiple flare factors is needed for clinical signs to develop (Hillier & Olivry, 2004). Because not all predisposed individuals develop CAD, the influence of environmental factors in the natural history of the disease cannot be ignored.

A threshold effect for clinical signs has been observed in affected cases of CAD, where a summation of pruritic stimuli appears to be needed for signs to occur

(Marsella & Sousa, 2001). Hence, an individual who is sensitized towards both HDM and pollen can be asymptomatic during long periods of the year because the mites alone are not enough to trigger pruritus. During pollen season however, the threshold might be crossed and the patient displays clinical signs of CAD. Other factors that can potentially contribute to an affected dog reaching its “pruritic threshold” are concurrent CAFR, flea bite dermatitis, secondary skin infections and environmental factors such as humidity and/or temperature. Furthermore, the “pruritic threshold” will vary between individuals and could potentially be lowered by stress (Marsella & Sousa, 2001).

### *Prevention*

If the purpose of investigating predisposing factors of CAD is to develop the means prevent disease, it is important to distinguish between primary and secondary prevention. Primary prevention aims at hindering the disease from developing in an individual and secondary at reducing clinical signs in affected patients (Arshad, 2005). A means of primary prevention in the canine population could be to avoid breeding CAD positive dogs, but this is complicated by the fact that the disease can debut after sexual maturity. If dogs at high risk of developing allergic disease could be detected through a diagnostic test at young age these individuals could be excluded from the breeding pool. Elevated chord blood levels of IgE in newborns have shown correlation to future development of allergy in humans, although the predictive value of the IgE level alone is weak (Allam et al., 2005). Regardless of CAD status, puppies have been reported to have low and adult dogs to have very high levels of plasma IgE (Ledin et al., 2006). Furthermore, no correlation between total serum IgE levels in young puppies and subsequent development of clinical signs of allergy has been detected in dogs and such testing is therefore of little diagnostic value (DeBoer & Hill, 1999). Genetic testing for early detection of atopic susceptibility is currently not available for neither canines nor humans, but could present a powerful tool for the development of prevention strategies in the future.

### *Clinical relevance*

CAD is a chronic disease that affects an unknown number of dogs. It will cause variable degrees of discomfort in affected individuals, who are also prone to other dermatological problems such as secondary skin infections and otitis externa. Understanding the epidemiology of CAD, including disease frequency and major risk factors, is important to veterinarians striving for better clinical management of affected cases as well as for owners and breeders aiming at prevention of the disease.

## **Atopic dermatitis in humans**

In the human literature, atopy has been defined as a genetic propensity to produce IgE antibodies after exposure to allergen, and allergy as clinical manifestations of immunologically mediated reactions to foreign substances (Arshad, 2005). Some of the clinical manifestations of allergy are asthma, allergic rhinitis, food allergies

and atopic dermatitis/eczema. Atopic patients tend to develop a spectrum of these manifestations through time. Typically, affected infants will display eczematous skin and gastrointestinal symptoms followed by asthma and rhinitis to inhalant allergens later in life. This time-dependent development has been termed “the atopic march” (Johansson et al., 2001).

### *Pre-disposing factors*

There has been a considerable increase in the prevalence of AD among children in industrialized countries over the past few decades (Leung & Bieber, 2003). Due to the high prevalence, the disease is a substantial public health problem in addition to its negative effects in affected families. Williams (2000) reviewed the epidemiology of AD and found the disease to be slightly more common in female children. Furthermore, a higher prevalence of AD has been seen with higher socio-economic status and smaller family size, which has been taken to suggest that a “western lifestyle” is responsible for the observed increase in prevalence. In a worldwide, cross-sectional questionnaire survey of the prevalence of AD, symptoms of the disease were found to be more common in Australasia and Northern Europe while the prevalence was lower in Eastern and Central Europe and Asia (Williams et al., 1999). Although there is a genetic background for the disease, it seems unlikely that the increasing prevalence in western, industrialized countries should be due to a shift in genetic make-up. Consequently, several authors point out that the increasing prevalence of manifestations of AD must be due to interactions between genes and the environment (Williams, 2000).

Rural living has been reported as a protective factor against the development of allergies in children in various studies (such as by Alfvén et al., 2006). Factors that might contribute to a higher prevalence of allergies among children living in cities are differences in environmental exposure to air pollution, home heating and allergens or lifestyle factors such as smaller family size and lower levels of exposure to livestock than in rural areas (Naleway, 2004). A recent cross-sectional study showed that consumption of unpasteurized milk had a protective effect against the development of AD and allergic sensitization in British children living in a rural environment (Perkin & Strachan, 2006). Perkin and Strachan (2006) conclude that farm living in itself does not affect the prevalence of AD, and suggest that the protective effect of rural living seen in other studies might be mediated through consumption of unpasteurized milk. In a comparison of Swedish and Estonian children, the observation was made that the prevalence of AD was lower, while circulating IgE antibodies more common, among the latter group (Voor et al., 2005). The authors speculate that the observed difference in prevalence could be due to Estonian children having more contact with various micro-organisms in early life, and hence a different development of the immune system as compared to children in Sweden.

### *Immunology and “The hygiene hypothesis”*

The so-called “hygiene hypothesis” was proposed by Strachan (1989), and the basic principle is that microbial exposure in early life influences the immune

system in a way that provides protection against the development of atopic reactions. The hypothesis is that the higher prevalence of allergic diseases, including AD, seen with decreasing family size could be due to fewer cross-infections from older siblings during childhood (Strachan, 1989). The rationale behind the “hygiene hypothesis” is that exposure to microbes will promote cell-mediated immunity and type 1 helper T cells ( $T_H1$ ), while the humoral immunity seen in newborns and dominated by type 2 helper T cells ( $T_H2$ ) is down-regulated.  $T_H1$  cells are associated with response to infection while  $T_H2$  are typically involved in allergic reactions mediated by cytokines that stimulate production of IgE and maturation of mast cells, eosinophils and basophils (Leung & Bieber, 2003). Recently, a more balanced view on the relationship between  $T_H1$  and  $T_H2$  responses in allergy has emerged, and the importance of T regulatory cells in the control of allergen-specific immune responses through the suppression of both lines has been documented (Akdis, Blaser & Akdis, 2005). In a review of the existing literature regarding AD and the hygiene hypothesis, Flohr et al. (2005) conclude that no specific pathogen appears to be linked to the “allergic”/  $T_H2$ -dominated immune-response. Rather, the authors suggest that the reported protective effects associated with early attendance at day-care, endotoxin and animal exposure are due to non-pathogenic microbial stimuli over time (Flohr, Pascoe & Williams, 2005). Alterations in gut microflora, specifically through increased colonisation with lactobacilli, is proposed as an immunomodulatory mechanism that will suppress the  $T_H2$  response through the pathway of T regulatory cells (Baker, 2006). This has given rise to new research where dietary supplementation of probiotics (=live microbial food supplements with proved beneficial effect on human health) in infancy has yielded decreased prevalence of AD (Rautava, Kalliomaki & Isolauri, 2005).

### **Comparison between canine and human atopic dermatitis**

Canine atopic dermatitis shares many similarities with the disease in humans. Pruritus is the main clinical sign in both species, combined with a familiar or hereditary predisposition and a limited extent of primary lesions (Rhodes, Kerdel & Soter, 1987). Furthermore, affected individuals from both species are susceptible to secondary skin infections and display similar histopathological features. House dust mites, in particular the species *Dermatophagoides farinae* and *D. pteronyssinus*, appear to be the most important indoor allergens in both species even though the specific peptides involved in the allergen reactions differ (Nuttall et al., 2006). CAD is a chronic disease, while an unknown proportion of human patients go into complete remission in their early teens. However, children with AD are at increased risk of developing respiratory allergies as well as skin disease later on in life (Thestrup-Pedersen, 2000). This so-called “atopic march” seen in human patients is not a documented feature of CAD. It should also be noted that two types of AD are described in the literature. The extrinsic type is associated with IgE and is most common, while the intrinsic type has no IgE mediated sensitisation (Leung & Bieber, 2003). Whether these two variants of AD differ in terms of severity, response to treatment or subsequent development of asthma is not known (Williams & Flohr, 2006). Other authors argue that non-IgE

mediated dermatitis should not be termed atopic in the first place, and some controversy exists regarding the nomenclature (Johansson et al., 2001). For the vast majority of patients with CAD, allergen-specific IgE can be demonstrated either by intradermal testing or by IgE serology.

Due to the many similarities, CAD can potentially serve as a spontaneous model for AD in humans and thereby increase our knowledge regarding the disease in this species (Hillier & Olivry, 2004). However, little is currently known about the epidemiology of CAD due to the problems discussed previously; few reliable studies, lack of uniform diagnostic criteria and possible misclassification bias. It is therefore of interest to perform studies aimed at determining if similarities exist between the epidemiology of atopic dermatitis among the two species. An overall aim of this thesis was to perform investigations into the incidence of and risk factors for CAD. It is further of interest to compare the findings to what is known about the epidemiology of AD in humans in order to evaluate the usefulness of this “canine model” for future comparative studies.

### **Estimating disease frequency in companion animals**

Unfortunately, a great deal of confusion regarding the terms incidence and prevalence exists in the veterinary literature. Due to limitations in study quality and availability, neither the prevalence in a general population nor the incidence of CAD is currently known (Hillier & Griffin, 2001b).

#### *Measures of disease frequency*

Incidence rate (IR) measures the number of new disease cases in a population per animal-time unit at risk (Dohoo, Martin, & Stryhn, 2003a). The time units can be expressed as dog-years/months/days at risk, and because they can be selected arbitrarily the numerical value of the IR has no direct interpretation. Incidence rates are used when the aim of a study is to evaluate what factors are related to a disease. The individuals who contribute time to the denominator of an IR are referred to as the “population at risk” (Rothman & Greenland, 1998a). For a chronic disease such as CAD, an individual will no longer contribute time at risk to the denominator after the disease has been recorded because CAD can only emerge once in an animal’s lifetime. The incidence risk, also known as cumulative incidence or incidence proportion, is the probability that an individual will develop a disease within a defined time period (Dohoo, Martin & Stryhn, 2003a). This measure is used when the objective of a study is to make individual predictions about disease occurrence.

Prevalence can be defined as the proportion of a population that has a disease at a specific point in time (Rothman & Greenland, 1998a). The prevalence of a disease is a function of its duration and incidence, and a disease with high incidence can have low prevalence if it is rapidly fatal. Prevalence is less useful than IR in evaluating risk factors for disease because it is affected by factors that contribute to either disease occurrence or duration (Dohoo, Martin, & Stryhn,

2003a). Because CAD is a chronic disease that rarely kills the affected individual, its incidence is expected to be lower than the prevalence in a stable population.

### *Sources of population data for companion animals*

Prevalence is calculated by dividing the number of diseased individuals with the total number in the population at the same point in time, while animal time-units at risk in the population constitute the denominator in the IR. The calculated measure of disease frequency therefore depends on the available study population. In general, IRs are rarely calculated in companion animal studies because the time at risk contributed by each individual in the studied population is seldom known. Studies of disease frequency in companion animals are often based on already existing data with its given limitations regarding quality, completeness and representability. Some recent examples of both primary (collected for research purposes) and secondary (already existing) sources of data for measures of disease frequency and risk factors among companion animals are presented below.

#### Primary data collection

Incidence rate calculations can be performed through cohort (or their variant longitudinal) studies, in which a group of presumably healthy individuals are followed over time to see who develops the disease in question (Dohoo, Martin, & Stryhn, 2003b). The IR of canine mammary tumours among three dog breeds in Norway was estimated by combining information from diagnostic tumour submissions and a census of dog owners of the breeds Bernese mountain dog, boxer and bichon frisé (Moe, 2001). Overall, cohort studies are rare in companion animal epidemiology.

In cross-sectional studies, exposure and disease status for a sample of subjects are determined at a given time and the obtained measure of disease frequency is the prevalence. This design is frequently applied in veterinary epidemiology (Dohoo, Martin, & Stryhn, 2003c). A questionnaire aiming at determining disease prevalence among purebred dogs in Denmark is an example of a cross-sectional study (Proschowsky, Rugbjerg & Ersboll, 2003). The response-rate was low in this study and the representativeness of the underlying population at risk unknown. It was concluded that diseases of the skin and ears were breed-dependent and affected 13.6% and 13.2% of the included dogs, respectively. Serological investigations in a group of animals are also examples of cross-sectional studies that will yield a prevalence of sero-positive individuals at the time of sampling (sero-prevalence). In a recent survey, the sero-prevalence of canine babesiosis and ehrlichiosis was determined at a veterinary teaching hospital in Brazil by testing a systematic random sample of its patients (Trapp et al., 2006).

#### Secondary databases

Other sources of information about canine populations are secondary databases, which contain data that was not originally collected for research purposes. Disease recordings from veterinary hospitals have been used extensively for these kinds of studies, and authors have collected information about diagnosis and animal



attributes from both single hospitals and combinations of hospitals in larger databases. Even for the larger studies there is a lack of knowledge about the population at risk, because only animals that come into the hospital are recorded and the underlying number of dogs that could give rise to the recorded cases remains unknown. However, these studies can be used to calculate hospital based prevalence of disease and to determine if for instance some breeds are over-represented with a diagnosis as compared to the general hospital population. In a recent study including 3707 small animal consultations from 20 general practices in the UK, 21.4 % involved a dermatological diagnosis and 4.7% of the dogs with a specific dermatological diagnosis were reported to have CAD (Hill et al., 2006). From a large study of 52 private veterinary practices in the US, the prevalence of CAD was reported to be 3.1 % among 31,484 examined dogs (Lund et al., 1999).

Claims records from animal insurance companies constitute a less commonly used source of secondary data on canine populations. The main advantage of these databases is that information on entry, exit, disease status and more (such as breed, gender, date of birth, postal address) for a large number of healthy dogs is included. Because the time at risk contributed by each individual is known, IR calculations can be performed. Data on insurance claims from a UK company was used to calculate the IR of canine neoplasia (Dobson et al., 2002). The incidence was age-standardized based on a survey of the general canine population because the insured dogs in general were younger, and more often pure-bred, than the background population of dogs in the country.

Several studies have been published in which the claims database from Agria ([www.agria.se](http://www.agria.se)), the main Swedish insurance company to offer veterinary care and life insurance for animals, has been used to study general as well as disease specific morbidity and mortality among Swedish dogs. Two specific examples are a study on breed risk of pyometra (Egenvall et al., 2001) and the incidence of mammary tumours among bitches (Egenvall et al., 2005). The skin (and integument) has been reported as the most commonly affected organ system for veterinary care claims in the database, with a total of 3.15 % of dogs having a reimbursed claim pertaining to this system during 1996 (Egenvall et al., 2000a).

This dissertation contains two studies of the incidence of and risk factors for CAD among Swedish dogs insured by Agria. It is important that issues regarding data quality are addressed when secondary databases are used to measure disease frequency, because the data were not specifically collected for this purpose. Furthermore, the representativeness of the study population in relation to a broader group of animals should be evaluated. Assessing the data quality of the diagnosis CAD in the insurance database was among the specific aims of this thesis.

## Aims

The overall aim of this work has been to increase the knowledge regarding the epidemiology of canine atopic dermatitis. By gaining further insight into the occurrence of, and risk factors for, the disease it will hopefully be possible to provide better care for affected individuals. Ultimately, a goal could be to reduce the incidence of CAD.

The following specific aims were set:

- To validate the correctness of the diagnosis CAD in a Swedish animal insurance database (I).
- To estimate the incidence rate of CAD among insured Swedish dogs (II).
- To identify high-risk breeds for CAD, as well as to quantify the importance of the risk factors age, gender, season of birth and geographic location (II).
- To analyze the spatial distribution of CAD cases in Sweden and relate spatial patterns to environmental risk factors for the disease (III).
- To identify predisposing factors for the development of CAD in the three high-risk breeds boxer, bullterrier and West Highland white terrier (IV).

## Methodological considerations

Overall issues regarding the methods applied in paper I – IV are outlined in this section. For detailed descriptions of materials and methods, the reader is referred to each individual paper.

### Study populations

Two different populations were studied regarding the epidemiology of CAD; a secondary database obtained from the Swedish animal insurance company Agria (I – III) and a prospectively collected case-control material (IV). The special characteristics of each population and implications for the performed studies are presented in the two following sections:

#### *Insured population*

The Swedish canine population is unique in that an estimated 70 percent of the animals are covered by an insurance plan for veterinary care and/or life, and that the largest insurance company (Agria) covers approximately 30 percent of the entire population (Egenvall et al., 1999). Egenvall et al. (1999) further conclude that the insured population is representative of the general canine population with the exception that mixed breeds and older dogs are less likely to be covered by an insurance plan. The quality of the diagnostic information included in this secondary database has been validated against practice records and deemed acceptable for research purposes (Egenvall et al., 1998).

The study population in papers I to III consists of dogs insured by Agria. The target (or reference) population to which the results are to be extrapolated is the general Swedish canine population, keeping in mind that the under-representation of older dogs and mixed breeds might negatively affect the ability to extrapolate from study to target population (defined as internal validity by Dohoo, Martin, & Stryhn, 2003d). This under-representation might not be a major problem when studying CAD because it is a diagnosis of young animals with a breed predisposition affecting mainly pure-bred dogs. External validity is defined by Dohoo, Martin & Stryhn (2003d) as the ability to extrapolate results to an even wider general population, beyond the target population of Swedish dogs. In this case, the general population could be other national dog populations. The genetic make-up of pure-bred dogs can differ between geographic regions and the high-risk breeds reported from the insured Swedish dogs might not be the same breeds as are seen in other countries.

An advantage of using the insured population when studying disease frequency and risk factors is that longitudinal information on a large number of (presumably) healthy dogs is available. Because the time of death is known for many individuals, survival after diagnosis can also be calculated. Due to the large size of the study sample (>220,000 dogs in papers II and III) the power to detect

differences in risk is higher than in many smaller studies. A disadvantage is that only information included in the database is available, and this is often quite limited as the data were not originally collected for research purposes. Furthermore, only treatments which exceed the set deductible will be eligible for reimbursement and hence generate a recorded diagnosis. This will contribute to an underestimation of the occurrence for diseases that do not require costly treatments or diagnostic work-ups.

### *Case-control population*

In paper IV, a prospectively collected case-control material was analysed. Three high-risk breeds for CAD were identified in the insured population (II) and confirmed by the literature; boxer, bullterrier and WHWT. Cases of CAD from these three breeds were collected by 12 veterinary dermatologists in Sweden, and non-affected controls matched by breed and year of birth were selected randomly from the Swedish Kennel Club's (SKC) registry. Standardized inclusion and exclusion criteria were applied in order to ensure uniformly defined cases. Information about each individual dog was collected from four sources; the SKC registry, owner questionnaires, clinical observations by the investigating veterinarian and a telephone-questionnaire to the dogs breeder. Furthermore, blood samples were collected for genetic analysis.

The main advantage of collecting this specific material was that the inclusion and exclusion criteria could be defined to ascertain the CAD status for all cases and controls. By retrieving information on early rearing from the breeders, risk factors that preceded the development of CAD in time could be recorded. This allows for claims of causality, which is usually not the case in cross-sectional and case-control studies where data on outcome and exposure are collected at the same time. A disadvantage of the design is that no inference can be drawn about the effect of breed or age on the risk of CAD, because these were used as matching variables. One might speculate that the owners and breeders of CAD cases could be more likely to remember exposures in the past that could be related to the development of the disease, also known as recall bias (Rothman & Greenland, 1998b). This was sought minimized by having both the interviewer and the breeder blinded to case-status when the telephone interviews were performed.

### **Validation of the diagnosis CAD among insured dogs (I)**

A subset of the CAD cases in the insured population studied in papers II and III was chosen for a validation of the diagnosis in the Agria database. Letters requesting medical records from all reimbursed CAD cases during 2002 were sent out to the attending veterinarians, unless a full medical record was available in the insurance company archive. The assumption was that the diagnostic criteria applied in the field had not changed substantially through the years 1995 to 2002, which was the included time-span for the analyses in papers II and III. The diagnosis was validated based upon available information from the collected medical records. Each individual was classified on an eight-point "CAD-scale" by

the principal investigator, reflecting how “correct” the diagnosis was considered compared to the pre-set diagnostic criteria.

## **Incidence rate calculation (II)**

The IR of CAD was calculated using exact denominators, because the time at risk contributed by all insured dogs was known. In addition to the overall incidence, the IR stratified by variables recorded in the insurance database could be calculated (ie IR by gender, breed, geographic region, age, year and season of birth). Furthermore, the proportion CAD cases out of all dogs with dermatology claims was estimated to allow comparisons with other published studies.

## **Risk factor analyses (II, III, IV)**

Individual dog-level risk factors for CAD were investigated among the insured population as well as in the collected case-control material. Furthermore, the IR of CAD was aggregated by postal-code area (PCA) and geographical risk factors were studied through spatial analysis. In the three different regression analyses performed, lack of independence between observations was taken into account using various techniques. The dependence was created by dogs belonging to the same breed (II), living in neighbouring PCAs (III) or being examined by the same veterinary practitioner (IV).

### *Cox regression with a random error term (II)*

Time until development of CAD was studied using a Cox proportional hazards model in paper II. The rationale behind applying the regression model was to be able to simultaneously control for several risk factors for CAD, and to determine their relative importance. Breed is a well established risk factor for the disease and had to be included in the model. Because the analyzed dataset included 130 different breeds, it was not practical to include a separate fixed effect for each breed in the model. Breed was instead included as a random effect, or shared frailty term (Dohoo, Martin, & Stryhn, 2003e). This implies that individuals belonging to the same breed were not independent of each other but rather considered at similar risk of developing CAD; they were equally “frail”. After building the final proportional hazards model, a ranking of breed frailties was produced and compared to the crude ranking of breed-risk of CAD based on IR.

### *Spatial analysis (III)*

Spatial analysis typically consists of the three steps visualization, exploration and modelling (Pfeiffer, 2004). For paper III, the IR of CAD among insured Swedish dogs was aggregated by PCA and initially visualized as a choropleth map. Spatially smoothed empirical Bayes (EB) incidence rates were subsequently calculated to minimize the effect of small sample size in some areas (Bailey & Gatrell, 1995). The presence of areas of increased or decreased risk was evaluated using global (Moran’s *I*) and local indicators of spatial association (LISA)

(Anselin, 1995). Finally, environmental risk factors were explored and a “risk map” was produced utilizing a spatial Poisson regression model (SAS, 2005). The model uses the x and y coordinates of the PCA centroids as a random error term to take the lack of independence between neighbouring areas into account. If there is a geographic pattern in the occurrence of CAD, it seems likely that observations that are close to each other in space would be more similar than observations that are further apart.

#### *Logistic regression with random effects (IV)*

A logistic regression model was used to investigate risk factors for CAD in the case-control study reported in paper IV. Forty different veterinarians were involved in the data collection and each contributed between one and 22 dogs to the study. Dogs that were recruited from the same veterinary practice were likely to share certain attributes, such as urban versus rural living, and examining veterinarian was therefore added to the logistic regression model as a random effect. The variable was not truly random because the 12 “main” veterinarians were selected based on their interest and expertise in veterinary dermatology. However, it was not deemed practical (nor relevant) to include 40 fixed effects for veterinarian in the model as these were nuisance parameters and not the focus of the analysis. Furthermore, examining veterinarians with only one dog included in the study were grouped together in the random effects model (n=27) and hence assumed to be the same. This is obviously a simplification, as large individual differences might exist within this group.

## **Results and discussion**

### **The importance of eliminating adverse food reactions (I)**

The practice records obtained in paper I were compared to a slightly modified version of Willemse’s (1986) criteria for CAD. The modification was justified by the fact that none of the suggested clinical criteria are considered perfect, and as long as transparency exists as to which criteria were applied it is still possible to compare results between studies. Of the scrutinized cases, all were recorded by the submitting veterinarian to be suffering from CAD and 98% were judged by the principal investigator as having allergic skin disease. However, for a large number of dogs CAFR had not been properly ruled out and in total it was suggested that approximately 75% of the cases had CAD or CAD with concurrent CAFR. Gastrointestinal signs were recorded more frequently in the medical records of individuals that subsequently responded to diet trials. However, this was not strong enough as a discriminating factor to utilize for predicting which of the animals that had not undergone such testing were likely to have CAFR.

In the examined insurance database, it was concluded that the correctness (whether the recorded cases actually have the disease) of the diagnosis CAD was

acceptable, while the completeness (proportion of diseased cases that were in fact recorded) was unknown but most likely low. Furthermore, all recorded cases were coded by the submitting veterinarian to have CAD and therefore the information was accurate regarding the transfer of diagnostic information from medical records to the insurance database. Internal validity was defined by Jordan et al. (2004) as whether the diagnosis perceived correct by the clinician is recorded in the database, and can in this case be classified as excellent. External validity was defined by the same authors as whether the patient has the disease coded by the examining practitioner, and relates to the diagnostic ability of the veterinarian as judged against an external standard (Jordan, Porcheret & Croft, 2004). By this definition, the external validity for the diagnosis CAD in the insurance database was questionable because some veterinarians did not appropriately exclude CAFR.

As stated previously, it is important to validate secondary databases if they are to be utilized in research. Papers II and III rely on the information obtained in paper I. The results from this study confirm that CAD is a subjective diagnosis and that the quality of the work-up depends on the individual veterinarian. Jordan et al. (2004) performed a systematic review of validation studies to assess the quality of human morbidity coding in computerized general practice records. It was concluded that conditions with clear diagnostic criteria, such as diabetes, overall had better quality recordings than subjective diagnoses such as asthma (Jordan, Porcheret & Croft, 2004). The presented results for CAD are in agreement with this finding, and this needs to be kept in mind when interpreting the results from paper II and III. Most importantly, the proportion of recorded CAD patients that should actually be coded as CAFR positive or with a dual diagnosis of CAD and CAFR remains unknown.

## **CAD incidence varies by breed (II)**

In paper II, the overall IR of CAD was calculated as 1.7 new cases per 1000 dog years at risk (DYAR). The 95% confidence interval (CI) for the estimate was 1.67 to 1.77 cases per 1000 DYAR. For a relatively rare occurrence like CAD, the yearly incidence risk can be directly extrapolated from the IR to be 0.17% which means that an individual dog's risk of developing CAD during a year is 0.17% (Dohoo, Martin, & Stryhn 2003a). The proportion of dogs with a recorded dermatologic diagnosis that were coded as having CAD was 5.1 %, which is in agreement with the result obtained by Hill et al. (2006) in Great Britain but is at the low end of the range of estimates published by other authors. It is believed that the true incidence of CAD is underestimated in the insured population due to the perceived low completeness of the database regarding the diagnosis.

The performed study confirmed that breed is an important risk factor for CAD. The bullterrier had the highest IR of CAD with 21 (95% CI 15.6 – 26.4) cases per 1000 DYAR. Other breeds at increased risk were the Welsh terrier, boxer and WHWT. Three of the top-risk breeds from paper II were selected for further sampling in paper IV. (The Welsh terrier was not included due to relatively few registered individuals of this breed in Sweden.) When looking at high-risk breeds

for disease from a national population, it should be noted that results cannot necessarily be extrapolated to other national populations because even purebreds might be genetically different across countries. However, several of the high-risk breeds presented in the current study have also been reported to be at increased risk by other authors, such as the Dalmatian, Labrador retriever, WHWT, boxer, German shepherd and bullterrier (Sousa & Marsella, 2001).

With the recent sequencing of the canine genome, new opportunities to search for disease-causing or predisposing genes arise (Lindblad-Toh et al., 2005). Due to years of selective mating, individuals within the same canine breed share homologous genetic regions to a much greater extent than humans. These conserved regions of the canine genome are useful for comparative studies of genetic diseases, because many of the genetic diseases seen in humans such as diabetes, cancers, epilepsy and AD are also present among dogs. Furthermore, it is likely that the same risk allele is carried by multiple related breeds. Lindblad-Toh et al. (2005) therefore conclude that genome comparisons within and across breeds can reveal genes that underlie inherited traits and that association studies for almost any disease can be performed with today's resolution of the canine genome sequence because of the high degree of homology. A subset of the included boxers and WHWTs were used to evaluate if a candidate gene for AD could be associated with CAD in dogs, but yielded negative results (Stenshamm et al., 2006). DNA collected from the presented cases and controls will be used for further genetic analyses in the future.

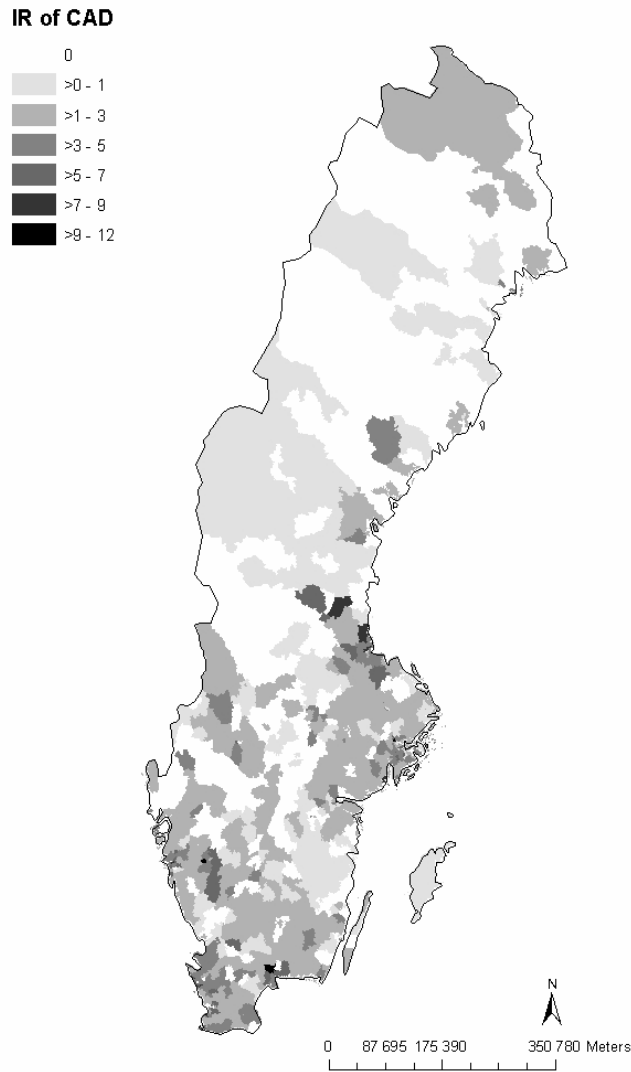
### **Spatial patterns in the distribution of CAD cases (III)**

The crude incidence rate of CAD by PCA is presented as a choropleth map in figure 1. Figure 2 shows the spatially EB smoothed IR of CAD. The overall trend is that the IR is higher in the southern parts of the country and in densely populated areas. This becomes more obvious in figure 2 because the smoothing process provides more stable IR estimates in areas with small populations at risk by "shrinking" the individual rates towards their neighbourhood means (Bailey & Gatrell, 1995).

Several factors were found to be associated with an increased risk of CAD through the spatial regression model; increasing human population density (HPD), increasing levels of average annual rainfall, the presence of a veterinary dermatologist in the county and living in the south of Sweden. Paper II also revealed city living as a risk factor for CAD, which is in agreement with the observation that the prevalence of AD among humans is increased with urban living. However, it was established in paper I that for a subjective diagnosis like CAD it will depend on the skills of the examining veterinarian whether a patient is correctly diagnosed or not. Many of the Swedish veterinarians that specialize in dermatology are located in densely populated areas and it cannot be ruled out that the effect of urban living in the current studies is an effect of practitioner rather than of the environment in the cities itself. The association between CAD and city living could not be confirmed in the case-control material when the effect of



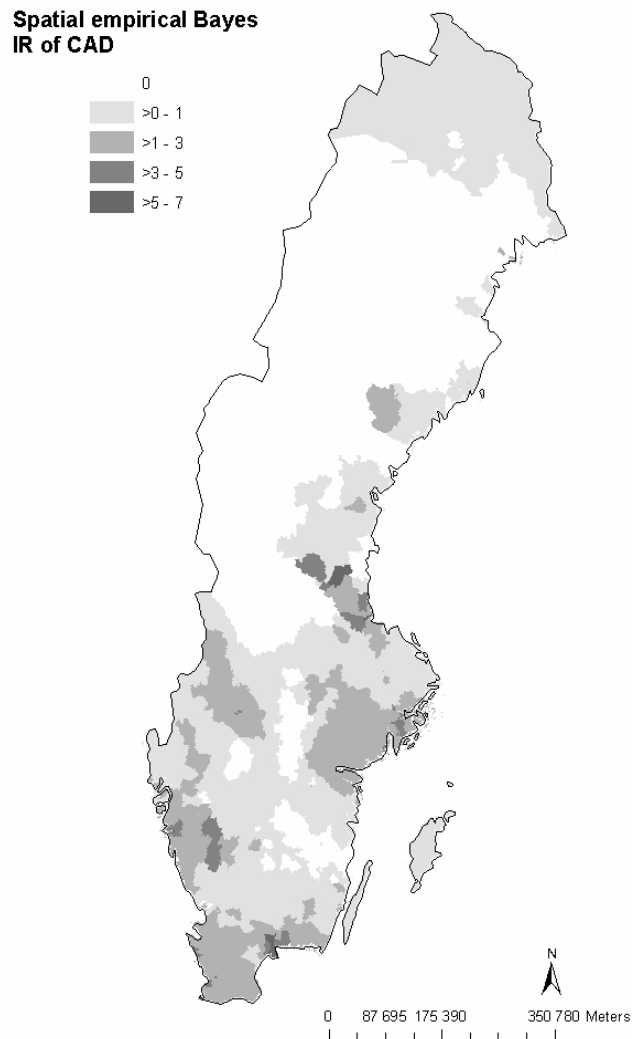
submitting veterinarian was controlled for, but the sample-size in this study was limited and there might have been insufficient power to detect a relationship.



*Figure 1.* The crude incidence rate of canine atopic dermatitis per 1000 dog years at risk in Sweden between 1995 and 2002, by postal code area (n=559).

The IR of CAD was lower for dogs living in the northern half of Sweden than in the south. In previous studies of overall morbidity (Egenvall et al., 2000b) and breed risk of pyometra (Egenvall et al., 2001), it was also concluded that disease occurrence was lower in the north. A possible explanation for this phenomenon is

that the canine population differs between regions in a way that favours “healthier” breeds in the north. It can further be speculated that there are less recorded claims in the north because there are fewer specialized veterinary clinics in this sparsely populated region of the country or that dog owners there are less likely to seek veterinary assistance. The spatial regression analysis was performed on IR data aggregated by PCA, and the breed or age-distribution of the population could therefore not be included in the model.



*Figure 2.* The spatial Empirical Bayes smoothed incidence rate of canine atopic dermatitis per 1000 dog years at risk in Sweden between 1995 and 2002, by postal code area (n=559).

Finally, an increasing risk of CAD was seen with increasing levels of average annual rainfall. It was speculated that this could be due to more patients reaching their “pruritic threshold” due to exacerbation of clinical signs with increasing humidity, and hence being subject to examination by a veterinarian to a greater extent (Marsella & Sousa, 2001). Furthermore, increasing humidity might provide better conditions for HDM which could then act as a trigger for the development of CAD (Arlian, Neal & Vyszynski-Moher, 1999).

#### **Early diet and its effect on CAD occurrence (IV)**

In paper IV it was shown that feeding a home-made/non-commercial diet to the bitch during lactation significantly decreased the odds of her offspring becoming CAD positive in the three high-risk breeds bullterrier, boxer and WHWT. This is an interesting finding from a comparative point of view. The recent adjustments in the understanding of the “hygiene hypothesis” and the importance of gut microflora for the modification of the immune response might be related to this effect of early dietary exposure (Flohr, Pascoe & Williams, 2005). The presented analysis did not include a breakdown of ingredients in the non-commercial diets, but it seems reasonable to assume that the content of micro-organisms in food prepared manually in the home would be higher than in commercial dog foods. It should be kept in mind that the diets did not have to consist exclusively of non-commercial ingredients to have a protective effect, the combination of home-made and commercial food also contributed to decreased odds of CAD.

Studies in humans have shown a lower prevalence of AD in children exposed to unpasteurized milk (Perkin & Strachan, 2006), in Estonian versus Swedish children (Voor et al., 2005) and in children from families with an antroposophic lifestyle (Alfven et al., 2006). Björkstén et al. (2001) performed a prospective study and concluded that intestinal microflora in infants can play an important role in protection against allergy. Based on these, and several other, studies it has been suggested that the  $T_H2$  response associated with AD can be down-regulated through exposure to non-pathogenic micro-organisms which has led to the recent focus on probiotics for primary prevention of the disease. However, the relationship between microbial exposure and development of AD is unlikely to be straightforward and issues like timing and magnitude of the exposure might modify the effect (Williams & Flohr, 2006).

Only the association with diet during lactation was analyzed in the regression model, and no conclusions can be drawn about the effect of home-made diets during pregnancy. It is also important to note that pregnant and lactating individuals have special dietary requirements that need to be fulfilled regardless of the origin of their diet. Further research, preferably randomized controlled clinical trials, is needed before recommendations can be made about changing the diet of the pregnant and/or lactating bitch to prevent CAD in her offspring.

## **Other pre-disposing factors (II, IV)**

No effect of gender on the occurrence of CAD was detected in either of the two examined populations (paper II and paper IV). Based on this finding it seems reasonable to exclude gender from further discussions on risk factors for CAD. However, it is important to remember that the vast majority of Swedish dogs are intact and some caution should be exercised in extrapolating the result to other populations where neutering is routinely performed.

Based on the analysis performed in paper II, it was confirmed that CAD occurs most commonly in young individuals. The hazard of a recorded CAD diagnosis was highest for dogs between one and five years of age. This interval might seem higher than the age of onset of six months to three years that has been reported in the literature (Griffin & DeBoer, 2001). However, due to complexity of diagnosing CAD there is likely to be a time-lag between first occurrence of clinical signs and a recorded CAD-diagnosis in the insurance database.

In paper IV, white coat-colour was found to be a risk factor for CAD among the included bullterriers. Because the factor could only be investigated for one breed that consisted of very few individuals, no firm conclusions can be drawn about the association between coat colour and risk of CAD at the current stage and more studies are needed to further elucidate this issue.

Papers II and IV include conflicting results regarding the effect of season of birth on the development of CAD. In the insured population (paper II), the risk of CAD was higher among individuals born during the fall months of October to December. Meanwhile, in the case-control population collected for paper IV no such effect was detected. In genetically predisposed humans, maternal exposure to allergen during late pregnancy can determine if the predisposition will be manifested as allergic disease in the offspring (Warner et al., 2000). Warner et al. (2000) further hypothesize that continued exposure to the same antigens in the newborn might reinforce this effect. Based upon these findings, it would appear that individuals born during seasons of high exposure to certain allergens could be at increased risk of developing an IgE response towards the same allergens. This issue needs to be investigated further for CAD.

## Conclusions

- The cases recorded with CAD in the Agria insurance database were all coded by the examining veterinarian to be CAD-positive. However, the diagnostic ability of some practitioners was questionable because adverse food reactions were not properly ruled out. Overall, around 75% of the cases were believed to have CAD.
- The incidence rate of CAD among insured Swedish dogs was estimated to 1.7 cases per 1000 dog years at risk (95% CI: 1.67 to 1.77). This estimate is probably conservative, due to the perceived low completeness of the insurance database regarding this diagnosis.
- Substantial variations in the incidence of CAD exist between breeds, with the bullterrier being at the highest risk in this material.
- It was confirmed that CAD is a diagnosis of young animals.
- No difference in the incidence of CAD was seen between genders in two Swedish populations of predominantly intact individuals.
- Conflicting results were achieved regarding the effect of season of birth on CAD incidence. In the insured population, an increased incidence was seen for individuals born in the fall but this finding could not be confirmed through the case-control study.
- Two studies suggest that geographical variations in CAD incidence exist in Sweden. Living in a densely populated or humid area was associated with increased risk of the disease. However, the results might be biased by the geographical distribution of different breeds of dogs in Sweden, or by the location of the examining veterinarian because of the subjective nature of the diagnosis.
- A preventive effect of feeding a home-made/non-commercial diet to the lactating bitch on subsequent development of CAD in her offspring was observed for the high-risk breeds boxer, bullterrier and West Highland white terrier.

## **Implications for future research**

New insight has been gained regarding the epidemiology of canine atopic dermatitis through the described studies. However, several areas have been identified in which further research is needed:

A formal validation of the diagnostic criteria for CAD should be conducted, including calculation of sensitivity and specificity.

Spatial analysis on non-aggregated data would be a valuable addition to the existing study on geographical risk factors for CAD because it would allow taking breed, age and actual distance to a veterinary dermatologist into account.

The evidence supporting season of birth as a risk factor for CAD needs to be further investigated, as conflicting results were seen between the case-control study and the incidence rate study based on insurance data.

Focused studies are needed in order to determine if white coat-colour, or genes regulating coat-colour, are relevant risk factors for CAD. Genetic studies to identify other genes (or genetic regions) of importance for the development of CAD among high-risk breeds might also increase our knowledge regarding the disease.

Randomized controlled clinical trials are needed to confirm the results from the case-control study suggesting that primary prevention of CAD can be achieved through modification of the diet used for bitches during lactation.

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