List of abbreviations

AD	atopic dermatitis
Acc	Accuracy
A.e.	*Ambrosia elatior* (ragweed)
A.g.	Alnus glutinosa (alder)
APC(s)	antigen presenting cell(s)
Aneg	agreements of the negative results
Apos	agreements of the positive results
ARF	adverse reaction to food (food allergy)
A. siro	*Acarus siro* (meal mite)
ASIT	allergen-specific immunotherapy
A.v.	*Artemisia vulgaris* (mugwort)
B	intradermal skin test
B.p.	*Betula pendula* (birch)
C.a.	*Corylus avellana* (hazel)
cAD	canine atopic dermatitis
CCR4+	T-cells that carry the receptor for TARC
CD	cluster of differentiation
DCs	dendritic cells
D. farinae	*Dermatophagoides farinae* (housedust /farina mite)
D.g.	*Dactylis glomerata* (orchard grass)
D. pteronyssinus	*Dermatophagoides pteronyssinus* (housedust mite)
E1	ELISA1: membrane strip ELISA
E2	ELISA2: microtiter plate ELISA
EFA	essential fatty acid
ELISA	enzyme-linked immunosorbent assay
FA	food allergy (adverse reaction to food)
FAD	flea allergic dermatitis
FcεRI	IgE molecule-binding receptor with high affinity
fe	feline epithelium
FN	false negative
FP	false positive
fm	feather mixture
gr	grass mixture

hu	human epithelium
ICAM	intercellular adhesion molecule
IDEC	inflammatory dendritic epidermal cells
IDT	intradermal skin test
IL	interleukin
INF	interferon
LC(s)	Langerhans' Cell(s)
L. destructor	*Lepidoglyphus destructor* (hay mite)
MC	mast cells
MHC	major histocompatibility complex
NC	negative correlation
NK cell	natural killer cell
NPV	negative predective value
OD	optical density
PC	positive correlation
P. notatum	*Penicillium notatum* (mold)
Ph.p.	*Phelum pratense* (timothy)
P-K testing	Prausnitz-Küstner testing
P.l.	*Plantago lanceolata* (plantain)
PNU	protein nitrogen unit
P.p.	*Poa pratensis* (bleu grass)
ppm	particule per ml
PPV	positive predective value
RI assay	radioimmuno assay
RNA	ribo-nuclein-acid
Q.r.	*Quercus robur* (oak)
Sarcoptes-IgE	*Sarcoptes*-specific IgE antibody
Sarcoptes-IgG	*Sarcoptes*-specific IgG antibody
SDS-PAGE	sodium-dodecyl-sulfate-polyacrylamide gel electrophoresis
SIT	specific immunotherapy
Sn	sensitivity
Sp	specificity
S. scabiei	*Sarcoptes scabiei var. canis*
TARC	thymus and activation-regulated chemokine
TC	total correlation

Th1	T helper 1 cell (IL-2-, IL-3-, TNFβ- és IFNγ-termelő Th-sejt)
Th2	T helper 2 cell (IL-3-, IL-4-, IL-13-, IL-15- és IL-6 termelő Th-sejt)
TN	true negative
TP	true positive
TNF	tumor necrosis factor
T. putrescentiae	*Tyrophagus putrescentiae* (copra mite)
T. mentagrophytes	*Trichophyton mentagrophytes* (mold)
U.d.	*Urtica dioica* (stinging nettle)
we	weed mixture
WHO	World Health Organisation

CONTENTS

1. Introduction and objectives

The skin is the largest organ of the body and the anatomic and physiologic barrier between animal and environment. It provides protection from physical, chemical, and microbiological injury, and its sensory components perceive heat, cold, pain, pruritus, touch, and pressure. In addition, the skin is synergestic with internal organ systems and thus reflects pathologic processes that are either primary elswhere or shared with other tissues. Not only is the skin an organ with its own reaction patterns; it is also a mirror reflecting the *milieu interieur* and, at the same time, the capricious world to which it is exposed.

It is commonly stated that in an average small animal practice approximately 20% of all cases are dermatological. Flea infestation, flea allergic dermatitis (FAD) and secondary superficial pyoderma are the most common causes (30-70%) of skin diseases in the dogs. Some of the pruritic cases will be obvious, presenting no diagnostic difficulty, for example a pruritic dog with a heavy flea infestation. Many cases, however, need the same kind of systemic approach that would be necessary to investigate a neurological or cardiovascular problem. It is need in the pruritic dogs as well, where the different causes of pruritus (ectoparasites, secondary bacterial and/or fungal skin infections and allergic skin diseases) are to be excluded and/or diagnosed step by step. The 15-40% of all dermatological cases are the most common hypersensitivity rections: atopic dermatitis (AD), flea bite hypersensitivity and adverse reaction to food (ARF). The reported incidence of canine AD varies from 3–15% of dog population. Incidence of adverse reaction to food varies from 1-5% of all skin conditions and up to 23% of cases of nonseasonal allergic dermatitis. Up to 75% of food-hypersensitive dogs have other concurrent allergies such as AD and FAD (Reedy et al. 1997, Scott et al. 2001).

The major consideration in the differential diagnosis of pruritic dermatosis in dogs is the *Sarcoptes*-infestation. It has remained a consistent problem over the years occuring with variable frequency. *Sarcoptes*-dermatosis is highly contagious infection but marked individual variation in disease expression with the possibility of asymptomatic carriers. Infection generally results from direct contact but sometimes by indirect contact with the origin of the disease remaining

obscure. There is a possible contagion to humans, where zoonotic lesion (pruritic papules on the trunk, arms and legs) are common. Ectoparasitic skin diseases other than *Sarcoptic acariosis* (otodectic dermatitis, cheyletiellosis, Pelodera dermatitis, louse infestation, etc.), or endoparasitic skin disorders (infestation with the zoonotic *Dirofilaria repens*) are also to be considered during the differential-diagnosis of the pruritic canine skin diseases (Scott et al. 2001).

Clinical manifestations of canine atopy were first reported 50 years ago, in a dog affected with seasonal allergic rhinitis (Wittich 1941). Twenty years later, another author described a dog with allergic conjunctivitis, increased tear production and pruritus (Patterson 1960). It was only in 1971 that the clinical signs of canine atopic dermatitis (AD) initially were published (Halliwell and Schwartzman 1971). Since that time, veterinary medical literature has abounded with several hundreds of articles and textbook chapters that most commonly summarized clinical and laboratory data, and less frequently reported various aspects of the pathogenesis of the disease. Unfortunately, these clinical papers usually were based on anecdotal or dogmatic informations, clinical trials generally were open, uncontrolled and comprised few patients, and pathogenic data were often conflicting. Such a situation led to the perpetuation of poorly verified dogmas (e.g. the concept of an inhaled route of allergen contact), insufficiently tested pathogenic hypotheses (e.g. the issues of a putative delta-6 desaturase deficiency or IgG_d reaginic antibodies in dogs with AD), or therapeutic recommendations that relied on evidence of insufficient grade. Regrettably, several reports of original studies were not published beyond the level of a meeting's abstract, thus precluding their review and analysis by independent scrutineers.

There is an increasing incidence of atopic diseases (asthma, allergic rhinitis and atopic dermatitis) in humans, especially in industrialized countries. Although there is a genetic predisposition to the development of these diseases, the rapid rise in incidence is suspected to be caused by environmental rather than genetic factors. Neither the incidence nor the prevalence of atopic dermatitis in the general canine population has been studied. As many of the environmental factors associated with the increasing incidence of atopic dermatitis in humans are consistently found in the environment of dogs, it would seem likely that a similar increase in the incidence of this disease would be occurring also in dogs. The increasing prevalence of asthma, allergic rhinitis and AD in affluent western societies has been closely linked to increased indoor allergen load, an increased exposure to noxious pollutants, decreased family size, decreased microbial load and exposure to infection at a young age, increasingly urbanized environment, and changing dietary habits (Boguniewicz and Leung 1998). In addition, the more widespread use of prophylactic treatments for parasitic infestations may increase the incidence of atopic diseases, since data suggest that parasitic infestations may be protective against the development of allergy (Hagel 1993; Lynch 1993), although this hypothesis has recently been questioned (Weiss 2000). Genetic make-up is also believed to increase susceptibility to atopic diseases (Boguniewicz and Leung 1998). However, the rise in prevalence of these diseases in a relatively short period of time suggests that environmental factors play a greater role than do genetic factors (Okudaira 1998). In an early report, the prevalence of AD in the canine population at large was estimated to be 15% (Chamberlain 1974). More recently, in textbooks, estimates of 3–15% (Reedy et al. 1997) and around 10% (Scott et al. 2001)

have been stated. In a recent study of 31484 dogs examined by veterinarians in 52 private veterinary practices in the USA, 8.7% of the dogs were diagnosed with atopic/allergic dermatitis, allergy or atopy (Lund et al. 1999). It is stated in textbooks (Reedy et al. 1997; Scott et al. 2001) that AD is the second most common cause of canine pruritus, after flea allergy dermatitis. The true prevalence of canine AD is difficult to determine as: (1) mild cases are often successfully managed with symptomatic therapy without a specific diagnosis being made; (2) some clinical manifestations of AD are not recognized by owners or veterinarians as being part of AD (e.g. chronic otitis, bacterial and *Malassezia* infections); and (3) there are no documented reliable methods to demonstrate that clinical disease is induced by allergen exposure in dogs with allergen hypersensitivity. Further factors that may contribute to an increase in the incidence of canine AD in pet dogs are: dogs are spending more time indoors thus increasing exposure to common indoor allergens such as the house dust mites; there is more wide-spread vaccination of puppies which may increase IgE antibody production (Frick and Brooks 1983); and the practice of internal and external parasite control by dog owners is more common.

The aim of the first review was to summarize recent developements in canine atopic dermatitis reported during the past 10 years. Concepts regarding to the pathogenesis of AD have evolved substantially, including mechanisms involved in the primary disease and the role of secondary cofactors. New findings have profound effects on the present approach to AD diagnosis and treatment.

Reports of multiple case-series of canine atopic diseases began to appear in the literature in the 1960s and early 1970s, and established inflammatory skin lesions (e.g. AD *sensu stricto*) as a manifestation of canine atopy. In these studies, presence of skin disease is typically reported, but specific clinical criteria are neither noted nor discussed. A study, published in 1967, reviewed 13 cases with positive Prausnitz–Küstner tests, and described reaginic antibodies similar to those found in human atopic individuals, but discussed little about the clinical signs exhibited by the canine patients (Rockey and Schwartzman 1967). Clinical criteria for diagnosis were not presented. In 1971, a report of a cohort of 60 dogs with AD described statistical analyses on signalment of these cases, but only general statements on clinical findings were made (Halliwell and Schwartzman 1971). In this report, nasal and ocular discharges were occasionally described, but asthma-like symptoms and coughing were not found. It was stated that clinical signs of canine AD were at least initially seasonal in 75% of patients, coinciding with various pollination seasons. A retrospective review of canine cases with AD seen at a private practice in Illinois between 1972 and 1974 reported that AD was diagnosed in 30.7% of the dogs with dermatitis seen at that clinic (Anderson 1975). Interestingly, in this study no patients were diagnosed with food allergy, and flea allergy was

identified in only 7.8% of the cases. Criteria for diagnosis of AD were not presented, though it was stated that the face, paws, axillae and flanks were characteristic sites of involvement. Another study published in 1978 reviewed findings of 230 cases of canine AD, all referred for the primary complaint of pruritus (Nesbitt 1978). Diagnostic criteria also were not presented, though all dogs underwent intradermal testing. Clinical signs were described as "often a history of foot licking, face rubbing, or axillary pruritus, or combinations thereof" and inflammation that was "often seen first on less protected areas of the body, such as the ventral portion of the abdomen". Concurrent flea infestation, contact allergy, or adverse food reactions were diagnosed in an undisclosed number of patients. Signalment data were provided, but only for a selected group of 139 dogs that subsequently underwent allergen immunotherapy. Thus, even though early case-series did not use rigid criteria for diagnosis of AD or other allergy in dogs, and provided only casual descriptions of clinical signs, it was during these decades that canine atopic diseases were recognized to affect primarily the skin (Anderson 1975; Chamberlain 1974; Chamberlain 1978; Schwartzman 1986; Halliwell and Gorman 1989;). The first published studies attempting to quantify the frequency of clinical signs by body region appeared in the 1980s (Scott 1981; Willemse 1983; Nesbitt et al. 1984; Willemse 1986). However, it is difficult to compare the results from these studies, because criteria for diagnosis of AD varied among investigators (and in fact were not always specified), other diseases such as adverse food reactions may not have been eliminated from consideration, and intradermal testing procedures were variable. These potential inaccuracies in case data may thus lead to questions regarding the actual diagnosis of some of the patients included in the series. Generally, cases diagnosed with AD exhibited pruritus, had history and physical examination data compatible with AD, and diagnostic tests were performed to rule out the presence of some other (often unspecified) pruritic diseases. In two studies, a diagnosis of AD required the presence of pruritus of the face, paws, and/or feet (Willemse et al. 1983; Willemse 1986). The 1980s ended with new clinical criteria proposed for the definitive diagnosis of canine AD (Willemse 1986). A later study that evaluated these criteria found that some were not statistically associated with AD, and reported that pruritus, pyoderma, breed predilection and conjunctivitis were not helpful differentiating features (Prélaud et al. 1998). The latter authors proposed erythema of the forefeet, pinnae, and muzzle as more helpful criteria, along with assorted minor criteria. Though such lists of clinical criteria are helpful in determining if a patient’s signs are consistent with AD, they are not completely reliable in confirming a diagnosis. Indeed, a combination of the three major criteria proposed by Prélaud et al. (1998) only yields 80% diagnostic sensitivity. Authors of a recent text stress the hazards of using only these lists of clinical signs for diagnosis (Scott et al. 2001). Dogs with other pruritic nonatopic diseases (in particular, adverse food reaction and scabies) could satisfy the criteria in some instances, thus emphasizing the necessity for ancillary diagnostic testing to

eliminate from consideration diagnoses other than AD. Eliminating the possibility of food allergy is particularly cumbersome, because only a well-performed hypoallergenic diet trial followed by rechallenge is effective in ruling out this differential diagnosis. Papers published prior to 1994 often did not report performance of a diet trial; when one was performed, it was typically for a period of 3 weeks rather than the currently-recommended 8 weeks (Rosser 1993). The further issue of poor client compliance with diet trials (Carlotti and Costargent 1994; Saridomichelakis et al. 1999) increases the chances that some patients in early reports may have had food allergy in addition to, or instead of, AD. Moreover, this issue is confounded by the possibility that, in some dogs, food allergens could lead to the development of AD lesions.

Intradermal testing has been practiced for decades in human and veterinary medicine as „golden standard" for diagnosing the casual allergen. The primary utility of intradermal testing is in the demonstration of IgE-mediated allergen hypersensitivity. Intradermal testing is regarded as a valuable tool in the demonstration of allergen-specific hypersensitivity when performed according to accepted guidelines.

The aim of the second study was to present a survey about the frequencies of canine AD and occurrence of characteristic features of the disease based on 600 IDTs in our country. There has not been published such a large survey about canine AD in Europe. Moreover a lot of parameters were examined in each case which allows us to state new, statistically proven aspects about AD.

In the early work on the pathophysiology of the atopic diseases in man, the antibody responsible was shown to differ in many respects from classical antibody, and was termed "reagin" (Coca and Grove 1925). Reaginic antibody was shown to be destroyed by heating to 56°C for 4 h, and was transferable to normal skin of the same species by intradermal injection, persisting at the site for> 48 h (Prausnitz and Küstner 1921). This phenomenon forms the basis for the classical test for the presence of reaginic antibody — the Prausnitz–Küstner or PK test. Exhaustive studies in the late 1960s and early 1970s established that IgE was the major, if not the sole antibody with reaginic activity in man (Ishizaka 1967; Bennich 1969). It has also been shown that allergen-specific IgG_4 levels are often elevated in patients with atopic diseases, and it has been suggested that this antibody may have both "blocking" antibody activity, and possibly, on occasions, reaginic activity (Boluda et al. 1997). However, the evidence for a significant pathogenic role for the latter sub-isotype in atopic diseases is unconvincing (Aalberse et al. 1996). The role of IgE in different diseases classified as "atopic" is, however, controversial. Although it is established that reagins play a pivotal role in allergic asthma and rhinitis, the situation in AD is less clear. The first detailed report of a dog suffering from AD, is attributed to Wittich (1941). This first clinical case study

clearly implicated a reaginic type of antibody. The affected patient suffered anaphylactic shock when undergoing intradermal testing. Furthermore, serum from the patient gave a positive PK test not only when transferred to a normal dog, but also upon intradermal injection into human skin. The ability to transfer reaginic antibody to the skin of a heterologous species was recently confirmed in a more detailed study (Lowenthal et al. 1993), and forms the basis for the development of the Fcε-RI assay for canine IgE using the cloned human α-chain (Wassom and Grieve 1998). It is first necessary to ask if the clinical manifestations of canine AD are allergen-driven. In the vast majority of cases, either positive intradermal tests or *in vitro* tests for allergen-specific IgE are demonstrable. On rare occasions, however, reactivity to allergen is not demonstrable in patients that otherwise appear to be suffering from classical AD. If the patient is truly suffering from AD, there are a number of possible reasons for the failure to demonstrate allergen reactivity.

The aim of the third and fourth studies was to develope and evaluate ELISA serology test methods which demonstrate the presence of allergen-specific IgE antibodies from canine sera diagnosing the causal allergens in canine AD.

Infection with *Sarcoptes scabiei* var. *canis* occurs commonly in dogs. It often causes a severe skin disease which is difficult to diagnose and to differentiate from other pruritic skin conditions (atopic dermatitis, adverse food reaction), particularly in the acute stage of infection. Using skin scrapings from affected body areas is not a sensitive method, as mites are found in only 22.8-50% of samples (Bourdeau et al. 2004). Histopathology of the affected areas is not specific either if mites are not found. The indirect way of diagnosing the disease is treatment with acaricidal agents (Scott et al. 2001).

The aim of the fifth study was to evaluate the Swiss scabies ELISA test (IMOVET sarcoptes) in the diagnosis of canine scabies and differential diagnosis of atopic dermatitis. We measured and compared the results of Sarcoptes-serology and allergy-serology (measurement of allergen-specific IgE for other mites) in clinical patients with *Sarcoptes* infestation and in allergic patients. The sarcoptes-specific IgE was measured to examine hypersensitivity reaction to the *Sarcoptes* mite.

In most dogs with AD, both elimination of offending allergens and prevention of contact with allergens are difficult to achieve and response to pharmacotherapy often is unsatisfactory. In these cases, the possibility of modulating the immunological response that results from allergen exposure is appealing. This concept has led to the development of allergen specific immunotherapy (SIT), also known as hyposensitization, desensitization or allergy "vaccination". Such therapy

results in a variety of immunological changes, none of which are perfectly correlated with efficacy. The precise mechanism, is thus, unknown. Because of the lack of evidence-based recommendations for immunotherapy usage in dogs with AD, the WHO guidelines for immunotherapy in humans could be extrapolated to provide general directions of use. For example, one could propose that immunotherapy be reserved for dogs: (1) with demonstrable and clinically-relevant allergen-specific IgE antibodies, (2) in which allergen contact is unavoidable, (3) with symptoms that respond poorly to antipruritic drugs, or in which cost or side-effects of therapy are unacceptable and (4) whose owners are ready to afford the time, expense and technical aspects of this regimen. However, such guidelines should be validated in appropriate controlled experiments. Finally, one should not forget that immunotherapy is the only treatment option available that has the potential to result in partial or complete remission of canine AD without the further need of additional anti-inflammatory drugs.

Although the precise mechanism of SIT is unknown, the aim of the sixth study was to review the recent suggested methods of actions, to summerize the practical application of SIT including conditions for maximal efficacy and the conditions affecting treatment efficacy; to introduce the SIT in Hungary and to evaluate these results.

2. Chapter I

Recent developments in canine atopic dermatitis – a review

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Introduction

Atopic dermatitis (AD) is one of the most common allergic skin diseases of dogs. This entity has recently been redefined as a 'genetically predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features. It is associated most commonly with IgE antibodies to environmental allergens' (Olivry and Mueller 2003). The prevalence of canine AD in the different breeds depends on the canine genetic pool and is different in the different countries (as in Hungary magyar vizsla is predisposed to AD) (Tarpataki et al. 2006).

During the past 10 years, concepts regarding the pathogenesis of AD have evolved substantially, including mechanisms involved in the primary disease and the role of secondary cofactors. These new findings have profound effects on the present approach to AD diagnosis and treatment. Management of AD now requires that we view the large number of available treatment options as tools, the challenge being to select which combination of tools will provide best long-term control for an individual patient. It is a question which factors are involved in the pathogenesis of the primary disease and how these might be mitigated, but at the same time, attention must be paid to equally important secondary cofactors that may promote, augment, or exacerbate the disease.

Genetic factors

AD is a genetically complex disease that develops with gene-gene and gene-environment interaction. Extensive epidemiologic evidence in humans demonstrates the genetic basis of this disease, and this evidence was supplemented recently by abundant molecular genetic studies that show strong association between the atopic phenotype and several different chromosomal regions and specific genes (DeBoer 2004). No sole genetic factor or locus explains AD in all patients, however, and it also appears that the proper genetic background is necessary but not sufficient to result in expression of AD. Environmental influences clearly affect whether a human with the genetic background for AD will become clinically atopic. Numerous studies document the higher prevalence of allergic diseases in regions of the world with higher standards of routine healthcare and hygiene. The "hygiene hypothesis" holds that greater exposure to infectious organism through more limited availability of healthcare and exposure to less-hygienic conditions tends to promote a Th1 bias

to immune system and a lower prevalence of Th2-biased hypersensitivity disease (DeBoer 2004).

Strong breed predilections, familiar involvement, and limited breeding trials have demonstrated that canine atopy is genetically programmed. The heritability of the development of high-IgE response to antigen immunisation has been documented in a colony of beagle dogs. In beagles, the high-IgE response appears to be a dominant trait, but the development of clinical dermatitis occurs in only approximately 40% of the offspring (DeWeck et al. 1997a; DeWeck et al. 1997b). A critical aspect of high-IgE induction is that immunisation with the offending allergen had to occur shortly after birth and, if delayed until 3 to 4 months age, might be ineffective (Zunic et al. 1998).

Pathogenesis

The specific pathogenesis of canine AD remains unknown at this time, but the remarkable similarities between the human and canine diseases at both epidemiological and clinical levels (Hillier and Olivry 2004), suggest that immunological mechanisms leading to lesion generation are likely to be comparable.

Hypotheses for the generation of canine atopic skin lesions, the acute phase

The following steps may occur sequentially, but they could overlap or occur concurrently in canine AD (**Figure 1.**): An epidermal barrier defect could facilitate the contact of environmental, and possibly microbial allergens with epidermal immune cells at skin sites that have been subjected to friction and trauma. Epidermal Langerhans' cells (LCs) capture allergens with antigen-specific IgE and migrate to the dermis and regional lymph nodes. Keratinocytes are activated and release chemokines and cytokines, presumably in response to signals from LCs and/or microbes. Allergen-specific IgE-coated dermal mast cells release histamine, proteases, chemokines, cytokines and other mediators. There is a rapid influx of granulocytes (neutrophils and eosinophils), allergen-specific Th2 lymphocytes and dermal DCs. Eosinophils are activated and degranulate upon exposure to inflammatory mediators and allergens. Th2 lymphocytes and mast cells release type-2 cytokines promoting IgE synthesis and eosinophil survival.

The role of IgE-mediated hypersensitivity

Though allergen-specific IgE has been classically associated with the disease, more components of the immune system appear to be important. The role of allergen-specific IgG, Langerhans' cells, T cells, and eosinophils, as well as changes in the inflammatory milieu with

chronicity, are also being recognised as important components of the disease process (Scott et al. 2001).

Genetically predisposed dogs absorb through the skin, inhale, and possibly ingest various allergens that provoke allergen-specific IgE or IgG production. Canine IgE is (1) not precipitated in the presence of antigen, (2) inactivated at 56°C, (3) not complement fixing, (4) antigenically similar to human IgE, and (5) capable of passively transferring atopic sensitivities to normal dogs Prausnitz-Küstner (P-K) testing. In the last 20 years, many aspects of the structure and function of IgE and its receptors have been elucidated along with mechanism for regulation of these pivotal molecules. To date, no specific functionality or pathogenetic roles have been uncovered for different isoforms of IgE (DeBoer 2004). The lack of elevated serum total IgE levels in atopic dogs is because normal dogs have very high total IgE levels as compared with humans because of parasite-induced IgE, and the relatively minute levels of allergen-specific IgE-though enough to create disease- are not enough to change total IgE levels. Serum IgE levels are lower in laboratory dogs that live in confined environments with limited exposure and strict deworming programs. IgE levels increase with age up to 4 years. These findings are compelling evidence for the pathogenic role by allergen-specific IgE in canine atopic disease. However, the absolute requirement for IgE is questioned due to several observation in human or canine atopics: (1) atopy has been recognised in patients with agammaglobulinaemia; (2) allergen-specific IgE cannot even be detected in many atopic dogs and normal dogs experimentally sensitised to allergens; and (3) abnormally increased serum IgE levels generally do not fluctuate consistently during exacerbation, remissions, or treatment. However, allergen-specific IgE may decrease in response to hyposensitisation.

It is a question whether or not AD is always IgE mediated. Importantly, physicians allergists now recognize two different forms of AD in humans. In the “extrinsic” form, there is a family history of allergy and positive skin and serum allergen-specific IgE tests. In the “intrinsic” form, patients have identical clinical symptoms and family history of allergy yet do not show positive results with the same “allergy tests” (Novak et al. 2002). The latter patients represent ~30% of all human AD incidence and raise the question as to whether AD is always purely IgE mediated. It has become abundantly clear that there is more to allergy than just IgE; this fact has also caused to reconsider the role of IgE-based allergy tests in the overall diagnosis of AD. Perhaps we should not expect that all dogs with a clinical diagnosis of AD will show positive results on an allergy test- other factors seemingly unrelated to IgE-mediated hypersensitivity may explain the clinical signs (DeBoer 2004).

It was hypothesised that aberrant immune response led to inappropriate allergen-specific IgE production, and the IgE-bound to mast cells in the dermis. On subsequent allergen exposure, mast cell degranulation ensued with release of mediators such as histamine, and clinical signs were thus

produced. Conventional conservative therapy included antihistamines and anti-inflammatory fatty acid supplements. Use of the H_1-receptor-antagonist drugs to block the effect of histamine as a mediator met with limited success, although the drugs and dosages used were extrapolated from human medicine and may not have been optimal for animal use. Likewise, using fatty acid supplements in an attempt to interfere with the arachidonic acid cascade and production of inflammatory prostaglandins and leukotrienes was minimally beneficial (DeBoer 2004). This limited therapeutic success could have been a warning that the pathogenesis of AD is actually more complex than previously thought, although clearly, IgE-mediated hypersensitivity remains an essential component of the pathogenesis of AD for at least the majority of canine and human patients.

Upon exposure to most foreign antigens, the usual or "normal" humoral immune response results in production of IgG antibody rather than IgE. A major determinant of which antibody class predominates is which one of two subsets of T-helper lymphocytes (designated Th1 and Th2) is dominant. These subsets are characterized by different profiles of cytokine release upon activation. Th1 activation, the "normal" response, results in release of cytokines such as INF-γ and IL-2, which act to promote IgG production. If Th2 cells are activated instead, they release IL-4, -5, -13, and other proallergic cytokines, which results in recruitment of eosinophils into the inflammatory site and induce Ig-class switching in lymphocytes to result in production of IgE rather than IgG. The factors that determine whether a Th1 or a Th2 response will predominate are complex but include both genetic and environmental influences. The skin of dogs with AD overexpresses IL-4 mRNA and underexpresses transforming growth factor-β compared with healthy dog skin, which indicates a Th2-based response (Nuttal et al. 2002).

Factors other than immediate-type hypersensitivity

IgE-mediated hypersensitivity represents only one component of a more complex pathogenesis for AD, the other important potential factors are the next.

Epidermal barrier function

It is now well established that allergens can be absorbed through the skin instead of, or in addition to, via inhalation. In some humans, alterations in lipid and ceramide compositions were identified in the stratum corneum, which is the uppermost layer of the epidermis that is critical to epidermal barrier function (DeBoer 2004). Decreased barrier function of the skin often is evaluated experimentally by measurement of transepidermal water loss. In many human AD patients, transepidermal water loss is higher, which implies decreased barrier function and leads to the possibility that allergens and irritants can more easily penetrate the skin. This concept has not been

extended to dogs. However, preliminary and empirical ultrastructural (i.e., electron microscopic) examination of the stratum corneum of atopic dogs revealed differences between normal skin and skin of AD patients (**Figure 2.**) (Inman et al. 2001). In the normal canine skin, the keratinocytes of the stratum corneum are arranged in regular, overlapping layers, and intervening lipid material fills the gaps between cells in a classical "brick and mortar" arrangement. In contrast, the stratum corneum of dogs with AD shows more limited and discontinuous "mortar" between the cells.

If an allergen can penetrate the stratum corneum, the question is that it is then capable of eliciting a hypersensitivity reaction. In dogs and humans, the answer is clearly yes. Direct evidence comes from so-called atopy patch-test model, in which concentrated allergen preparations are applied to skin under an occlusive patch for periods of 24-48 h or more. In dogs sensitive to house dust mites, application of dust-mite allergen under the patch leads to skin lesions that histologically resemble those of AD (Olivry et al. 2002). Moreover, lesions consistent with AD can be induced merely by exposure of a sensitised dog to an allergen that has been painted onto the kennel surface on which the dog sleeps (Olivry et al. 2004). Thus in an individual who is already genetically susceptible to developing IgE-mediated hypersensitivity, reductions in epidermal barrier function that allow for allergens to enter the skin may contribute to the pathogenesis of AD.

The clinical implication of reduced epidermal barrier function also include those related to treatment. Limiting cutaneous contact with allergens may be helpful to these patients. This may include avoiding contact with the relevant allergen to the extent possible, also, more frequent rinsing or washing of exposed areas to remove allergens before they can penetrate the skin. Restoring or maintaining epidermal function may become an important part of therapy for AD. The latter could in theoretically be accomplished through topical applications that in some way restore the limited permeability of stratum corneum (DeBoer 2004).

Cutaneous antigen-presenting cells

IgE is present on the surface of mast cells, but importantly, it is now widely recognised in humans and dogs that IgE also has an important role and presence on the surface of cutaneous antigen-presenting cells (APCs). APCs, which are also called dendritic or Langerhans' cells (LCs), are cells of monocyte-macrophage lineage that function to capture and process foreign antigens and present them to the immune system, thereby initiating an immunologic response. The cells migrate among the epidermal keratinocytes, and upon encountering a foreign antigen, take the antigen in, process it by "digesting" it into smaller pieces, transfer these epitopes to the cell surface, migrate to the local lymph node, and "present" the allergen to the immune system by direct contact with lymphocytes. In part, the nature of the immunologic response that results is related to the type of receptors present on the APC. APCs that express IgE on their surface serve to focus the immune

response toward IgE production and immediate-type hypersensitivity. In both atopic humans and dogs and in both lesional and nonlesional skin of these AD patients, there exist a greater number of IgE-bearing APCs and greater expression of the IgE receptor on their surfaces; additionally, the prevalence of such cells correlates well with serum IgE levels (Olivry et al. 2004).

Epidermal Langerhans' cells in lesional skin of dogs with AD are hyperplastic, and are commonly seen in clusters. These LCs frequently exhibit surface-bound IgE, especially when they are aggregated. Epitheliotropic lymphocytes in lesional atopic skin possess either alpha-beta (αβ) or gamma-delta (γδ) T-cell receptors, and they express CD8 more often than CD4.

The role of keratinocytes

The cells of the epidermis itself (keratinocytes) are now recognised as rich sources of substances that may augment the inflammatory response. When keratinocytes become activated due to trauma, ultraviolet light, irritants, or other causes of skin inflammation, they release more than 30 different cytokines and related molecules (DeBoer 2004). These substances not only recruit inflammatory cells into the skin, but also stimulate APC activity and migration.

Keratinocytes in lesional skin of dogs with AD express the adhesion molecule ICAM-1, which allows CD11a-expressing leukocytes to bind to these epidermal cells. In the same areas, keratinocytes can express class II major histocompatibility (MHC II) molecules, a marker of activation. Immunohistochemical studies of keratinocytes in lesional atopic skin have also demonstrated intracellular staining for inflammatory cytokines (e.g. tumour necrosis factor-alpha-{TNF-α}) and chemokines (e.g. thymus and activation-regulated chemokine {TARC}, CCL-17).

Activated keratinocytes also release antimicrobial peptides (β-defensins and cathelicidins), which are an important part of the skin's ability to resist infection. It is now documented that skin cells of human AD patients may have reduced defensin production, and thus may allow colonization of and infection by bacteria (such as staphylococci) much more readily (DeBoer 2004). Although this observation has not yet been extended to animals, the parallels between human and canine skin with regard to staphylococcal infection and allergy are striking.

Low numbers of epidermal neutrophils are seen in approximately half of skin sections of canine lesional AD skin. Intact and degranulated eosinophils are occasionally detected singly or aggregated in subcorneal microabscesses (Olivry et al. 2004).

The role of secondary cofactors

Staphylococcal infection

It has been always recognised that cutaneous infections, particularly staphylococcal infections, are persistent and recurrent in canine AD patients. These secondary infections are emerging as important cofactors in the pathogenesis of AD. Often, the concept that the dog was allergic to the Staphylococcus bacteria was put forth as an important contribution to pruritus, and at one time, even intradermal testing with crude staphylococcal antigens was a common procedure. In theory, if dogs developed IgE against staphylococcal antigens, and the IgE subsequently sensitised mast cells, upon re-exposure to the staphylococcal antigen, degranulation and mediator release would occur and be accompanied by pruritus and inflammation. Because histamine can inhibit some functions of canine lymphocytes, there was speculation that inhibition of local immunity could occur and perpetuate the infection. In dogs, it was demonstrated that staphylococcal antigens could penetrate the stratum corneum, and that serum of patients with recurrent skin infections sometimes contained detectable anti-staphylococcal IgE (Olivry 2004). However, cause and effect has not been proven, and despite some efforts, the concept of staphylococcal hypersensitivity remains an unproven theory in dogs; if it does exist, it probably does not represent a major allergic mechanism.

In humans, staphylococcal exotoxins functioning as superantigens have emerged as important contributors not only to clinical signs but also to induction and maintenance of the allergic response, and these molecules may hold the key to the relationship between staphylococcal infections and AD. Staphylococcal toxins induce potent, direct, non-specific activation of lymphocytes, resulting in cytokine production and amplification of the inflammatory response in skin (Leung 2001). Moreover, they appear able to directly modulate the immune system toward allergy in human studies; for example, exposure of peripheral blood lymphocytes from human AD patients to staphylococcal toxins results in upregulation of IgE synthesis in cells, which is an effect that does not occur in lymphocytes from healthy individuals. Canine studies in this area are to be expected.

Regardless of the exact nature of this relationship, the clinical implication are profound. Infection alone may account for 50-90% of clinical signs in some people and pets with AD as is evidenced by sometimes-dramatic responses to antibiotic treatment. Early evaluation and treatment of staphylococcal infections are crucial in treating AD, and long-term infection control is a critical part of lifelong AD management. Options for longer-term control of staphylococcal infections include topical antimicrobial treatments, or intermittent use of oral or subcutaneously injectable antibiotics.

Yeast dermatitis

Regarding patient comfort, the role of overgrowth or infection of atopic skin with *Malassezia pachydermatis* yeast has no doubt been underestimated in the past, and merely recognising and treating it can a dramatic difference in some canine patients. The presence of IgE against yeast allergens is well documented in cases of human AD. It is known that some dogs with AD show a positive response to yeast allergens upon intradermal testing, and some dogs have yeast-specific IgE in their serum. The recent demonstration during passive-transfer experiments that serum from affected dogs can transfer reactivity to normal dogs is primary evidence that IgE-mediated *Malassezia* hypersensitivity exists in dogs (Morris and DeBoer 2003). Moist or greasy skin with severe pruritus, especially pruritus that is not responsive to glucocorticoids, should always prompt the clinician to check for yeast by cytology. Commonly affected areas include the interdigital spaces, axillae, inguinal region, and ventral neck, and skin in these areas may be prominently lichenified and hyperpigmented. The number of yeast found on cytology appears to be unrelated to how severe the clinical signs are; rather, it may be the degree of hypersensitivity that is important. Thus the finding of even one yeast organism on skin cytology of an animal with compatible clinical signs is justification for a treatment trial with topical or oral ketoconazole. Some AD patients have a 50-90% reduction in clinical signs just from treatment for *Malassezia* (DeBoer 2004).

Chronic phase

Several lines of evidence in humans suggest that the character of the cutaneous inflammatory response in AD may change over time. In studies using the atopy patch test, there is a clear predominance of Th2 lymphocytes infiltrating the area early in the course of the lesion; however, over time, with sever, chronic lesions, the pattern changes to a Th1 predominance (Olivry et al. 2004). This suggests a fundamental change in the nature of the inflammatory response in early vs. chronic disease. The clinical implications of this finding are important: early AD and chronic AD may in essence be two different diseases with different responses to treatments and differing prognoses.

Microbial overgrowth may contribute to chronic inflammation by producing polyclonal T-lymphocytes-activating superantigens, by activating keratinocytes and LCs through pathogen-associated molecular patterns and Toll-like receptors, and/or acting as allergens. Fibronectin and other molecules are exposed, which serve as sites for adherence of *Staphylococcus* bacteria to the skin and facilitate skin colonization by this organism. Self-trauma and neuromediators contribute to chronic inflammation. Pruritus resulting from the acute phase inflammation leads to self-trauma and release of neuromediators, both of which may cause further tissue damage and exacerbate

inflammation. There is a cycle of chemokine release (both allergen-dependent and allergen-independent) with subsequent further influx and activation of leukocytes, which in turn results in the release of additional chemokines and other mediators. There is an infiltration of T lymphocytes secreting type-2 and type-1 cytokines. Many of these lymphocytes are specific to initial offending allergen(s), but other T cells may also be recruited and stimulated non-specifically. Normal regulatory mechanisms, which could include regulatory T lymphocytes, fail to inhibit cutaneous inflammation.

Exposure of "self" antigens in the milieu of an active inflammatory response can lead to generation of antibodies against these components; autoantibodies against both epidermal and dermal components have been reported in human patients with AD (Valenta et al. 1999). The antigens are exposed in the vicinity of a great number of IgE-loaded antigen-presenting cells. These may then evoke an IgE autoantibody response, which in essence is the development of a hypersensitivity response against the self. Thus the inflammatory response may be initiated by environmental allergens, but with chronicity, it may be maintained by autoallergens. In this case, even complete removal of the environmental allergens may not create remission of chronic disease. From a clinical standpoint, adverse events occurring with chronicity argue strongly for early intervention and treatment of AD, when the disease may be more manageable and the potentially irreversible chronic changes have not yet occurred.

Therapy

Human and veterinary dermatologists and allergists use a variety of therapeutic modalities to provide lifelong management for their patients. Historically, AD is a disease that is most effectively treated using a combination of different therapies. Only now is it getting clear to appreciate from a pathogenetic standpoint why this is true.

Immunologic intervention

Allergen-specific immunotherapy (ASIT or "desensitisation") is an AD treatment wherein extracts of allergens to which the patient is sensitive are injected, in gradually increasing amounts over time, in an attempt to lessen or reverse the hypersensitivity state. The mechanism by which ASIT produces clinical benefit revolve around modulation of T-cell function and shifting the immune response from a Th2 bias to the more normal Th1 bias. Interestingly, most literature reviews state that ASIT is ineffective or at least questionably effective in human AD; in contrast, ASIT is considered by most veterinary dermatologists (Griffin and Hillier 2001) and the author's experience (Tarpataki et al. 2004) to be a highly desirable and useful treatment for canine AD.

ASIT acts on the IgE-mediated component of AD, which may explain why it works only partially or not at all in some patients.

Pharmacological intervention

Despite the fact that only a few pathogenetic observations have been extended to canine AD, on the therapeutic side, most new medications for human AD are fairly rapidly subjected to at least empirical trials in dogs. As new therapeutic targets have been uncovered, many new medications for control of allergic disease in humans have been developed. Unfortunately, very few of these have proven useful in dogs. Leukotriene receptor antagonists, 5-lipoxygenase inhibitors, and the nonsedating antihistamines have not been shown to be effective in animals (Olivry and Mueller 2003). Medications such as pentoxifylline and misoprostol may provide some relief in a few dogs (Olivry and Mueller 2003; Olivry et al. 2003). The newest treatments for canine AD such as the calcineurin inhibitors cyclosporin A and tacrolimus are aimed at interference with action of ILs and other cytokines. These drugs are highly effective at suppressing the allergic response and appear to have minimal adverse effects even when given over a long period of time (Stefan et al. 2003). Cyclosporin A administered at 5 $mg*kg^{-1}*d^{-1}$ successfully controls about 70% of atopic dogs, which is a level of control comparable to treatment with glucocorticoids (Stefan et al. 2003). Interestingly, in dogs with AD, improvement of clinical signs of AD was unrelated to cyclosporin blood levels.

Nutritional intervention

Nutritional considerations in AD used to be limited to the role that food hypersensitivity may play a role in a few patients. However, in the future it may be possible to influence the course of the disease with diet even in the absence of food allergy. It is clear that supplementation of the diet with fatty acids (EFA), for example, can change the lipid composition of the canine epidermis. In theory, this principle could be used in an attempt to augment compromised cutaneous barrier function. It may even be possible to prevent expression of the atopic phenotype in genetically predisposed individuals. For example, perinatal administration of probiotics such as *Lactobacillus* cultures to human infants at genetic risk for development of AD substantially reduces the risk that clinical disease will develop (Kalliomaki et al. 2001).

It has been seen suggested that AD (at least in humans) is the clinical result of a constellation of pathogenetic mechanism that may differ from patient to patient and over time in the same patient. Canine atopic dermatitis represents a substantial diagnostic and therapeutic challenge over a patient's lifetime, no single treatment being universally effective.

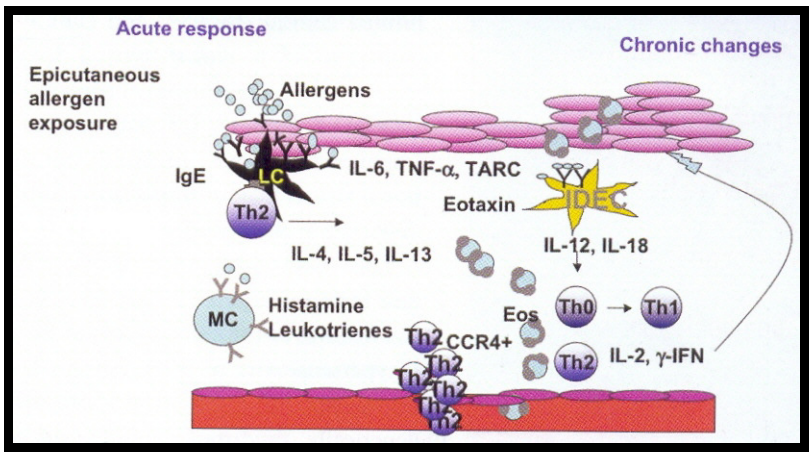

Figure 1. In the acute phase of the reaction, IgE facilitates the capture of the allergen. T-helper 2 cells promote IgE synthesis and eosinophil survival. Keratinocytes release proinflammatory cytokines and chemokines (e.g., eotaxin and TARC), which promote the recruitment of eosinophils and lymphocytes. As IL-18 and IL-12 are released, the T-cell population is more charactized by a T-helper type I response, which further promotes inflammation and epidermal changes. (Courtesy of: Rosanna Marsella, University of Florida, USA)

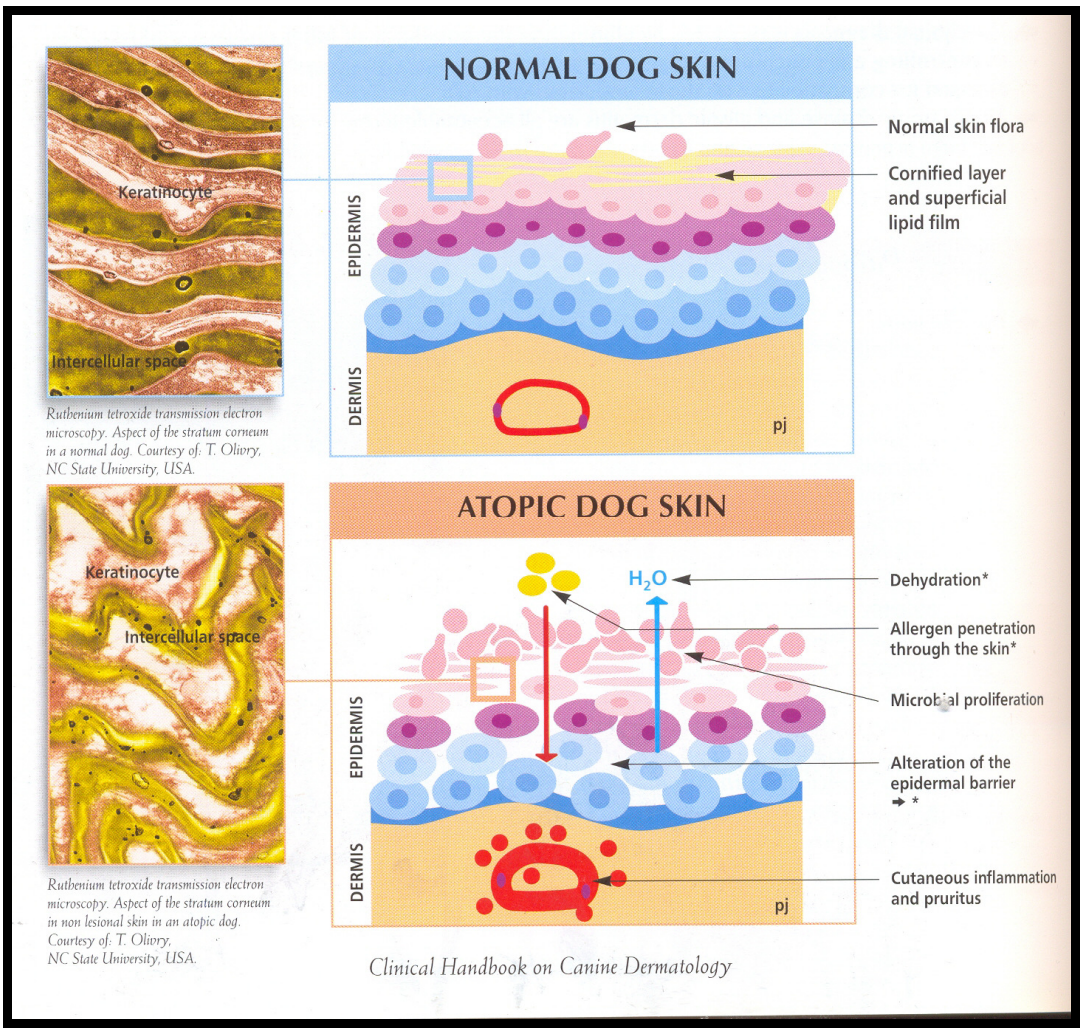

Figure 2. Ruthenium tetroxid transmission electron microscopy and the sketch of it. Aspect of the stratum corneum in a normal dog (Figure above), and in a non lesional atopic dog (Figure below) (Courtesy of: Thierry Olivry, NC State University, USA)

3. Chapter II

Prevalence and features of canine atopic dermatitis in Hungary

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Introduction

Diagnosis of AD has to be established by the owner's history, the basis of typical clinical findings and exclusion of other diagnoses (Willemse 1986; Carlotti 2004). The diagnostic criteria of Willemse are traditionally arranged in the so-called major and minor criteria. If 3 major and 3 minor criteria are established, and diagnoses other than atopy (pyoderma, flea allergic dermatitis, Sarcoptes-infestation, adverse reaction to food and all the other pruritic skin diseases) have been ruled out, the diagnosis of AD can be made. Diagnostic criteria of Carlotti (2004) are grouped into major and minor criteria too, but they are somewhat different from Willemse's (**Table 1**). Namely, the best compromise for diagnosis of AD in the practice is where three major criteria of Carlotti are satisfied. Considering the criteria, the greater the number that is satisfied, the more specific will be the diagnosis (but lower will be the diagnostic sensitivity).

Then the identification of offending allergens will logically be the next step. For that purpose serological tests or intradermal skin testing (IDT) can be choosen. To generate such data has relevance only, if clinical diagnosis of atopic dermatitis had been established prior to the testing, because large number of clinically healthy dogs have an increased level of allergen-specific IgE antibodies, too.

In Hungary, IDT has been used for more than 10 years in order to identify causing allergens in canine AD. Serological tests have been routinely available at least only for two years.

The aim of this work was to present a survey about the frequencies of canine AD and occurence of characteristic features of the disease based on 600 IDTs in our country. There has not been published such a large survey about canine AD in Europe. It is important for the small animal dermatologist's knowledge to have a picture about this region, too. Moreover a lot of parameters were examined in each case which allows us to tell/state new, statistically proved aspects about AD.

Table 1.

Diagnostic criteria for atopy in dog according to Willemse (Willemse 1986) and Carlotti (Carlotti 2004)

*Major criteria of Carlotti: corticosteroid responsive pruritus (at least at the beginning); otitis externa; inflammation of the internal surface of the pinnae (**Figure K**).

Minor criteria of Carlotti: seasonal aggravation of symptoms; variation in severity with environmental changes; aggravation when in contact with the grass (may also result in contact dermatitis) (Figure B, C**).

Major criteria of Willemse	**Minor criteria of Willemse**
Family history	*Onset before 3 years of age
**Breed predisposition	*Facial erythema and cheilitis (**Figure A, E**)
*Pruritus	**Bilateral conjuctivitis (**Figure E, J**)
*Facial and /or digital involvement (**Figure A, E**)	**Superficial pyoderma (bacterial folliculitis) (**Figure H**)
Chronic or chronically recurrent dermatitis (for more than two years) (Figure H, I**)	Hyperhidrosis
Lichenification of the flexor surface of the tarsal joint And/or extensor surface of the carpal joint (Figure F**)	*Immediate skin test reactivity to airborne allergens (**Figure D, G**)
	*Increased allergen-specific IgE concentration
	*Increased allergen-specific IgG concentration

Materials and Methods

Dogs (n=600) with subacut or chronic pruritus or reccurent pyoderma were examined by intradermal skin test (IDT). All dogs were referred by veterinary surgeons to dermatology ordinary of the Department and Clinic of Internal Medicine, Faculty of Veterinary Science, Szent István University, Budapest between 1999 and 2003. After taking nationale, history and performing examinations the same data were collected. The breed proportion and gender ratio were compared to the dog population of Budapest in 2000 (Bende et al. 2003). Breeds and breed groups were examined, too. Skin scrape samples were taken from all the dogs examined in the study. The 8 week-long elimination monodiet and the 3 weeks provocation diet to prove the adverse reaction to food (ARF) was prescribed if the patient had not had it before. Prior to skin testing all anti-inflammatory drug therapy, including oral and topical glucocorticoids and anti-histamines, were discontinued for at least 3 weeks and 10 days, respectively. IDTs were made by allergen solutions manufactured by Artuvetrin Laboratory (Artu Biologicals, P.O. Box 612, 8200 AP Lelystad. The Netherlands, www.artuvetrin.com). The applied allergens (and their frequences) are shown in the **Figure 5**.

Calculation of statistical significance was based on Pearson correlation analysis and/or chi-square test by SPSS Version 12.0 for Windows.

Results

Dogs (n=600) were examined by IDT in Hungary between 1999 and 2003. Seventy-eight (13%) cross-bred/mongrel and 522 (87%) thoroughbred/pedigreed dogs were represented with 79 breeds. The following breeds were overrepresented (**Figure 1**): 57 (9.5%) German shepherd dog, 36 (6%) Hungarian Vizsla, 31 (5.2%) Cocker spaniel, 26 (4.3%) Boxer, 24 (4%) West Highland white terrier, 22 (3.7%) English setter, 22 (3.7%) Poodle. The prevalence of IDT positivity in different breeds of the present study is listed in **Table 2** compared to dog population in Budapest.

There were 316 (52.7%) males, 7 (1.2%) castrated, 238 (39.7%) females and 39 (6.5%) spayed dogs in our examinations. Their ages varied from 4 months to 12 years.

The dogs were distributed according to their origin to four geographical regions of the country (**Figure 2**).

There were 308 (51.3%) indoor, 88 (14.7%) outdoor, and 204 (34%) indoor and outdoor kept dogs. Twentynine % of the patients lived together with other dog(s), 22% with cat(s) and 5.5% with bird(s). Seventyfive % of the patients were fed with mixed food, 16.6% with home-cooked food only and 8.4% with dry or tin food only. The main components of the food were chicken (92.2%), beef (84.7%) and pork (78.3%).

Table 2.

Prevalence of IDT positivity within breeds, compared to dog population in Budapest

Breed	**Number of dogs**	**Positive IDT reactions**	**Percent (%) of the + IDT**	**Percent (%) of patient population**	**Percent (%) of breed in Budapest's dog population**
German shepherd	57	35	61,4	**9,5**	**16,7**
Hungarian Vizsla	36	22	61,1	**6**	**3,6**
Cocker spaniel	31	14	45,2	**5,2**	**5,9**
Boxer	26	21	80,8	**4,3**	**0,9**
West Highland white terrier	24	14	58,3	**4**	**1,3**
English setter	22	14	63,6	**3,7**	**0,9**
Poodle	22	10	45,5	**3,7**	**5,6**
Dachshund	19	6	31,6	**3,2**	**8,4**
Chow Chow	17	8	47	**2,8**	**0,5**
Doberman Pinscher	17	10	58,8	**2,8**	**2,53**
French Bulldog	16	12	75	**2,6**	**0,2**
Dalmatian	16	9	56,3	**2,6**	**1,4**
American Staffordshire terrier	11	9	81,8	**1,8**	**1,7**
Newfoundland	10	9	90	**1,7**	**0,5**
Pumi	9	4	44,4	**1,5**	**0,77**
Bobtail	8	6	75	**1,3**	**0,7**
Puli	6	5	83,3	**1**	**2,8**
60 other breeds	1-5	0-100		**0,2-0,8**	

The examination of skin scrapings verified *Staphylococcus intermedius* in 58.4%, *Malassezia pachydermatis* in 28%, *Demodex canis* in 5.8% and *Sarcoptes scabiei var. canis* in 1.2% of cases. There were ctenocephalosis in 59 (9.8%) of all patients, the tipical dorsocaudal localisation of skin symptoms for flea allergic dermatitis was seen in 140 (23.3%) patients but only 25 (4.2% of the IDTs) gave positive reactions to flea saliva extract. Threehundred-thirty dogs (55%) improved during the 8 week long elimination monodiet, but only 55 (16.7%) of them had verified ARF after the provocation diet. The majority (87%) of the food allergic dogs were sensitive to chicken and 37% of them to a dry and/or a tinned food.

The majority (44%) of the examinations were performed in autumn, 21%-21% of the examinations were in summer and winter, and lower number examinations were made in spring (14%). The symptoms began within 1 year before the examination in more than the half of the cases (58%). The symptoms started in one third of cases before 1 year of age and one third between 1 and 3 years of age; in two third of the cases in spring and summer and in one third of the patients in autumn and winter. Pruritus was non-seasonal in 70% of the dogs. Drugs applied before examination against the symptoms of atopic dermatitis and their efficacy are demonstrated in **Figure 3**. The clinical symptoms and their prevalence are listed in **Figure 4**.

IDTs were positive for one or more allergens in 373 cases (62.2%) and 3 major and 3 minor symptoms of Willemse were established in 286 cases (47.7%). The majority (272; 72.9%) of dogs with positive IDT were allergic to *Dermatophagoides farinae* (*D. farinae*) house dust mite, 117 (31.4%) were sensitive for human dander and 97 (26%) to *Dermatophagoides pteronyssinus* (D. *pteronyssinus*). Weeds (30;8%), common mugwort (*Artemisia vulgaris*) (29;7.8%) and oak (*Quercus robur*) (25;6.7%) were the most common among seasonal allergens (**Figure 5**). Numbers of positive reactions of the 373 positive IDT results are demonstrated in **Figure 6**. There were 52 positive reactions to cat epithelia, but only 17 (32.7%) of them considered to be relevant since there were no cat(s) held together in the other positive cases.

Correlations

In the followings, all reported correlations and associations were found to be significant ($p<0.05$), but for better readability, p values are not displayed.

Geographic location

Dogs living in the garden suburb of Budapest were more sensitive to house dust mites, flea and molds opposite to the dogs from Transdanubia, the western part of Hungary that were most sensitive to weeds.

Gender

The 24.4% of females started the symptoms between 6 and 12 months; 61% of them at less than 2 years of age, the spayed females between 2 and 3 years of age, and the castrated males between 3 and 4 years of age. Males gave more positive IDT reactions than females did. Males gave more positive reaction to dust mites contrary to females. Fortythree percent of castrated males showed symptoms of seborrhoea oleosa. Spayed female animals had more often paraproctitis and females showed much more facial erythema than males did.

Age

Under one year of age the occurence of verified ARF, demodicosis, urticaria, folliculitis and gastrointestinal signs (recurrent diarrhoea, flatulance) was higher than in older ages. Between 4 and 5 years of age seborrhoea oleosa, positive reaction for flea by IDT were more common than in other ages. Dogs under 3 years of age began the symptoms in winter and spring, opposite to dogs older than 3 years that started their symptoms in summer and autumn. Dogs older than 6 years answered less to elimination monodiet, gave less positive reactions by IDT, especially to the *D. farinae* than younger ones.

Breed

German shepherd dogs began symptoms mostly between 5 and 6 years of age, and were most sensitive to Stinging nettle. *Hungarian Vizsla* started the symptoms in most cases between 6 and 12 months of age and 83.3% of them answered the elimination monodiet. Vizslas had more often otitis externa, conjuctivitis and facial erythema than other signs. *Cocker Spaniels* started clinical signs most often over 6 years of age, they had much less positive IDT rections and had filled more rarely Willemse's criteria than others. Cocker Spaniels answered the elimination monodiet well, and 22.6% of them were positive to ARF. Seborrhoea sicca and upper respiratory-signs (sneezing, nasal discharge) were more common in this breed than in others. *Boxers* started the clinical symptoms typically under 6 month of age, and much less over 3 years of age. Boxers answered the elimination monodiet well and they showed in most cases criteria of Willemse and IDT positivity, specially to house dust mites, molds, human dander and feather. The familiar occurence of AD, conjuctivitis, facial erythema, lichenification in Boxers was higher than in other breeds. The 79% of *West Highland white terriers* had verified ARF, they showed characteristic facial erythema and Willemse-major criteria. They were more frequently positive to feather than to ather allergens. Most of *English setters* started the clinical signs between 2 and 3 years of age and

were more frequently positive to woolen and timothy than to others. *Poodles* were most often positive to timothy and willow in opposite to dust mites. They had typically seborrhoea sicca. *Duchshunds* started their symptoms most often between 4 and 5 years of age, answered to elimination monodiet the least, and did not give positive reactions by IDT, even to house dust mites. *Dobermann Pinschers* had seborrhoea sicca and demodicosis more frequently than other skin symptoms. *French Bulldogs* started the symptoms mostly under 3 year of age, were more common positive to ARF, to feather and to wattle than to other allergens. Both (*French and English*) *bulldogs* were in most cases positive to ARF, molds and grasses. *Bulldogs* most often filled criteria of Willemse, had often conjuctivitis, hyperhidrosis, urticaria and *Staphylococcal* pyoderma. *Dalmatians* filled Willemse–criteria frequently, were more often positive to weed-pollens and grass-pollens, in opposite to house dust mites positivity. *Dalmata*'s symptoms were more frequently facial erythema and urticaria than other signs. *American Staffordshire terriers* started their symptoms usually between 1 and 2 years of age than in other ages, had more often verified *Sarcoptes*-infections, facial erythema, lichenification, symptoms in the elbow and groin region than other clinical signs, were more frequent positive to *D. farinae*, Common dandelion and to elimination monodiet. *Labrador and Golden Retrievers* were more often positive to grasses.

Breed groups

Correlation between breed groups and different aspects of clinical signs are demonstrated in **Table 3**.

Clinical signs and test results *(Figures A-L)*

Three major and three minor criteria of Willemse's were mostly true in Boxers, Dalmatians and French Bulldogs, but the IDT gave most often positive reactions in Boxers only.

Verified ARF occured most frequently in Cocker Spaniels, French Bulldogs, Bullmastiffs, Bull terriers, St. Bernard and Tervuren, and in West Highland white terriers and American Staffordshire terrier.

Table 3

Correlation between breed groups and different aspects of clinical signs. All reported positive and negative correlations and associations are significant ($p<0.05$), but for better readability, p values are not displayed.

Breed groups	started their symptoms under 3 years of age	started their symptoms over 3 years of age	answered to the elimination monodiet	positive reactions to provocation diet	positive reactions to grasses	Cteno-cepha-losis
Hungarian breeds	0	0	+	+	+	0
Sheepdogs	-	+	-	-	0	+
Terriers	+	-	+	0	0	0
Scent hounds	-	+	-	-	0	0
Sighthounds	-	+	-	-	0	0
Hunting dogs	0	0	+	+	0	0
Toybreeds	0	0	+	+	0	0

"+": positive correlation,

"0": no correlation,

"-": negative correlation.

Examined breed groups were the next:

Hungarian breeds: Hungarian vizsla, puli, pumi, mudi, kuvasz, Transylvanian Hound

Sheepdogs: German Shepherds, Beaucerons, Briards, Shetland Sheepdogs, Collies, Bobtails, Border Collies, Bearded Collies, Tervuerens, Polish Mountain Dogs

Terriers: West Highland white terriers, Yorkshire terriers, Cairn terriers, Scottish terriers, Wirehaired fox terriers, Boston terriers, Bull terriers, American Staffordshire terriers

Scent hounds: Basset Hounds, Beagles, Blue Gascony Hounds, Italian Hounds, Transylvanian Hounds, Griffons, English Foxhounds

Sighthounds: Afghan Hounds, Irish Wolfhound, Greyhounds, Hungarian Greyhounds, Italian Greyhound

Hunting dogs: Spaniels, Setters, Retrievers, Pointers, Vizslas

Toybreeds: Pekingeses, Shi-tzus, Lhasa Apsos, Bologneses, Havaneses, Poodles, Chihuahua, Bulldogs, Pugs, Spitzs

Discussion

Certain breeds are known to have a predilection for canine atopy (Scott 2001), including Boxers, setters (Gordon, English (Griffin 1993) and Irish setter's, terriers (West Highland white terriers, Yorkshire terriers, Cairn terriers, Scottish terriers, Wirehaired fox terriers, Boston terriers,

Bull terriers, American Staffordshire terriers), German Shepherd dogs, Chinese Shar peis, Chow Chow, English bulldog (Koch 1996), Lhasa apsos, Shi tzus, Dalmatians, Pugs, Golden retrievers, Labrador retrievers, Cocker spaniels, Poodles (Willemse 1983), Miniature schnauzers, Belgian Tervuerens, Chihuahuas, Shiba inus, and Beaucerons (Scott 2001). In Denmark canine atopy is seen most frequently among German shepherd dog, Newfoundland, Terriers, Boxers, Dalmatian, Shar-pei, Golden retriever, Labradors, American bulldogs, Setters, Lhasa apso's and Pekinese (Kristensen 2002). Only Kristensen (2002) suggested that AD can be seen in all breeds, although the disease is very rare in e.g. Doberman Pinsher, Rottweiler, Chihuahua and Pomeranian dogs. In the present study, the breed distribution was in agreement with the breeds listed above, except for Hungarian Vizsla, Pumi, French Bulldog, Dobermann Pinsher and Bobtail which were over-represented among atopic dogs compared to that breed distribution in Budapest. Although Puli was not over-represented 5 of the 6 Pulis gave positive IDT reactions. Nobody has previously examined and mentioned yet the AD and ARF in Hungarian breeds.

Canine atopy is reportedly more common in females than in males (Scott 2001), though a study showed no sex predilections (Willemse 1983). In this study males were over-represented, but it does not mean sex predilection if it is compared to the dog-population in Budapest where this ration is the same. What new in the present study is that males gave significantly more ($p<0,01$) positive IDT reactions than females did.

The age of onset of canine AD found in this investigation was not similar to that described previously (Scott 2001) because 66,6% of our dogs began their symptoms earlier, between 4 months and 3 years of age opposite to the age between 1 and 3 years of age. An exception to this general rule are Akita, Chow Chow, Golden retrievers and Shar Pei breeds, as mentioned in the literature (Scott 2001), and in the present study the Boxers too, wherein the signs of atopy may begin as early as 2-6 months of age.

The sesonality of clinical signs was higher (30%) then in the literature (20%) (Scott 2001).

In Hungary, the three most frequent positive reactions among non-seasonal allergens occured to *D. farinae* house dust mite (272; 72.9%), human dander (117; 31.4%) and *D. pteronyssinus* (97; 26%). Regarding the sequence of allergens these results are similar to others' in Europa, USA and Japan (Vollset 1985; DeBoer 1989; Sture 1995; Masuda et al. 2000; Mueller et al. 2000), where one of the house dust mites and/or human dander are most common. But the proportion (%) of positive reactions to human dander was lower and that of house dust mites were higher in our study than the results of those of most veterinary reports (Vollset 1985; DeBoer 1989; Sture 1995; Masuda et al. 2000; Mueller et al. 2000; Zur 2002).

Considering the reactions to pollen extracts, it is not possible to compare the present results with those of most veterinary reports because of the differences in geographic distribution of

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pollens between Hungary and other countries (Reedy et al. 1997). In comparison to human Hungarian studies the frequencies of pollen-allergy found in our study were similar (Magyar 2000). Unfortunately, the weeds-pollens (Common mugwort, Common dandelion and Common ragweed) play an important role in human and canine atopic diseases in Hungary.

In judging the relevance of positive skin reactions, it is necessary to correlate these reactions with the history of exposure. The irrelevant reactions to animal dandruff extracts, which was high in this study in case of cat epithelia, might indicate future atopic reactions, as they reflect the presence of skin sensitising antibodies only at the moment of testing. In addition, these reactions might be the result of cross reactivity among similar dandruff proteins (Willemse 1983).

Clinical signs and their occurrence are in accordance with other data described in the literature (Vollset 1985; DeBoer 1989; Sture 1995; Masuda et al. 2000; Mueller et al. 2000; Zur 2002).

Several results of the correlations described in the chapter 'Results' are not compared with other data from literature, because – as to the authors' knowledge - these results have not yet been published by others. The knowledge of these data may help the clinical management of AD and the breeding of atopic dogs. It is important to know for example that Dalmatians have got significantly higher positive reactions to seasonal allergens (grasses) than to house dust mites, because in general there is association between the month of birth and the incidence of canine atopy. Dogs born during the onset of pollen season more commonly suffered from atopy than control dogs (Zunic 1998). This finding suggested that dogs may be particulary susceptible to primary sensitization during the first 4 months of life, which was supported by the study about the high-IgE beagle model. Birth during nonpollen season would tend to decrease the incidence of sensitization. This could be a point of breeding for all breeds (German shepherd, Poodles, bulldogs, American staffordshire terriers, Labrador and Golden Retrievers, Hungarian breeds) which were significantly higher positive to seasonal allergens.

Breeds which usually have verified ARF (Cocker Spaniels, French Bulldogs, Bullmastiffs, Bull terriers, St. Bernard and Tervuren, West Highland white terriers and American Staffordshire terrier) are better to be fed hipoallergenic diet so as to be able to prevent clinical signs of ARF.

The authors hope they could help the small animal dermatologists with this large survey in Hungary to have a picture about canine AD in this region, too.

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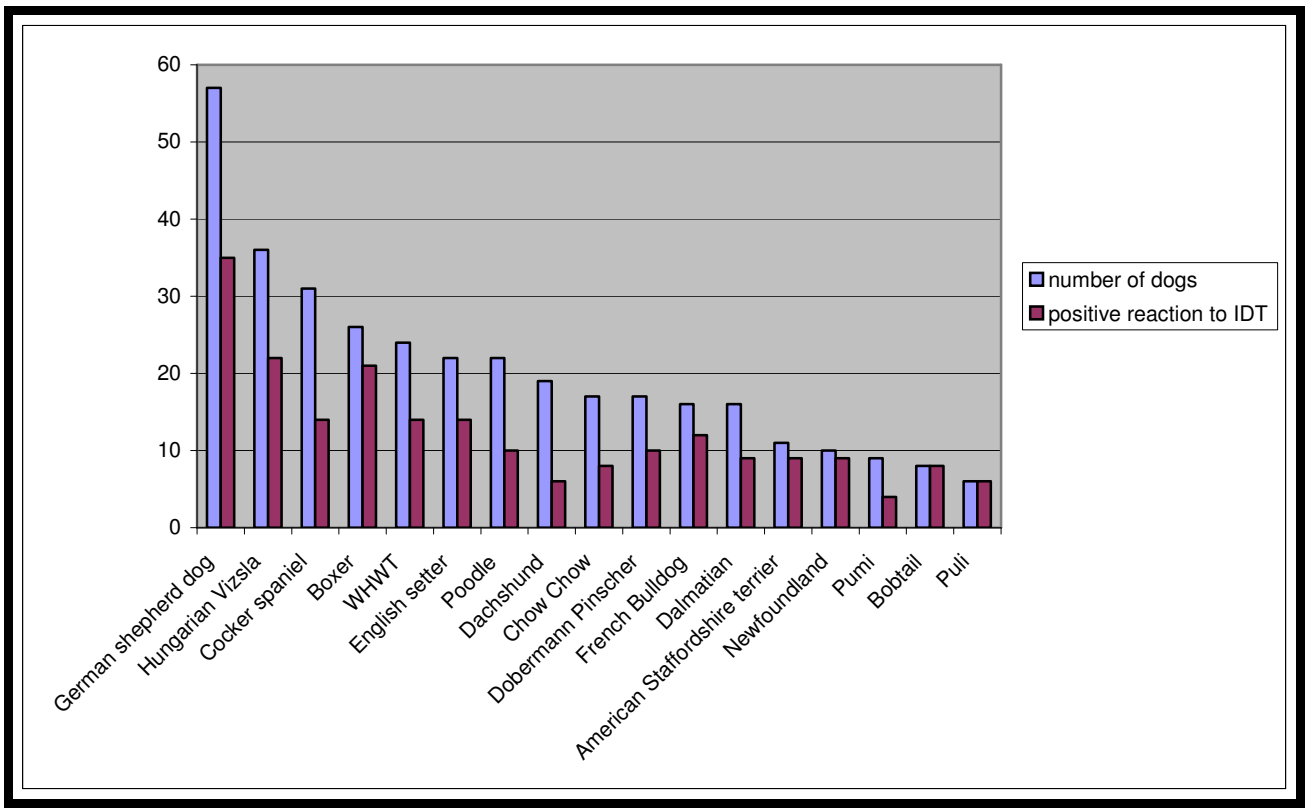

Figure 1. Number of positive IDT reactions in the different breeds.

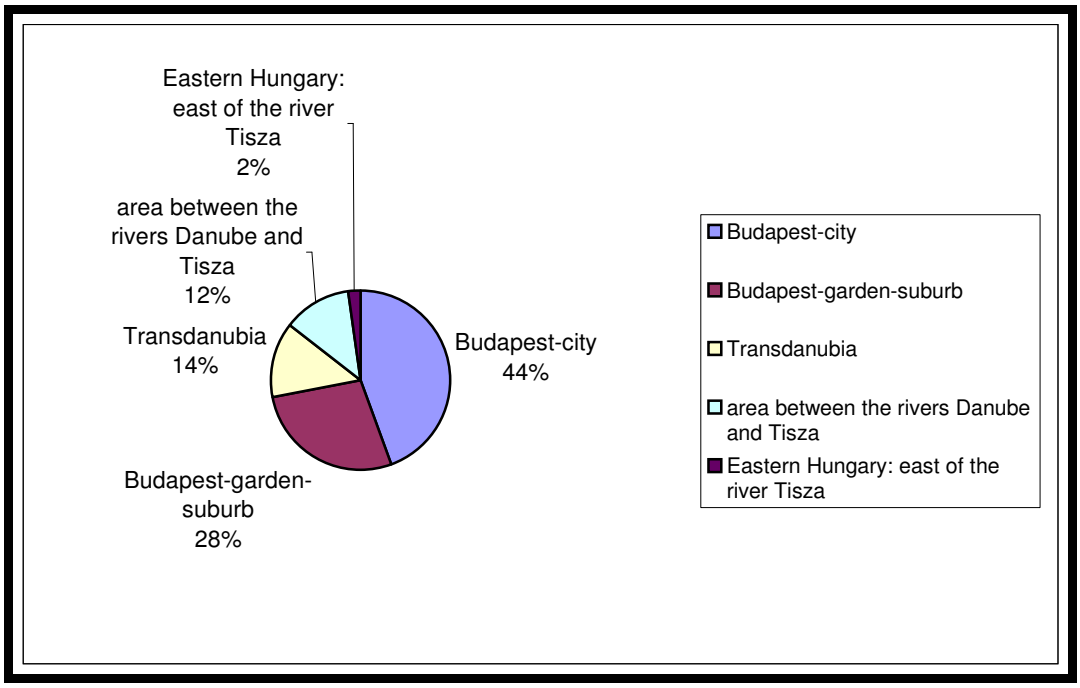

Figure 2. Geographical distribution of the examined dogs.

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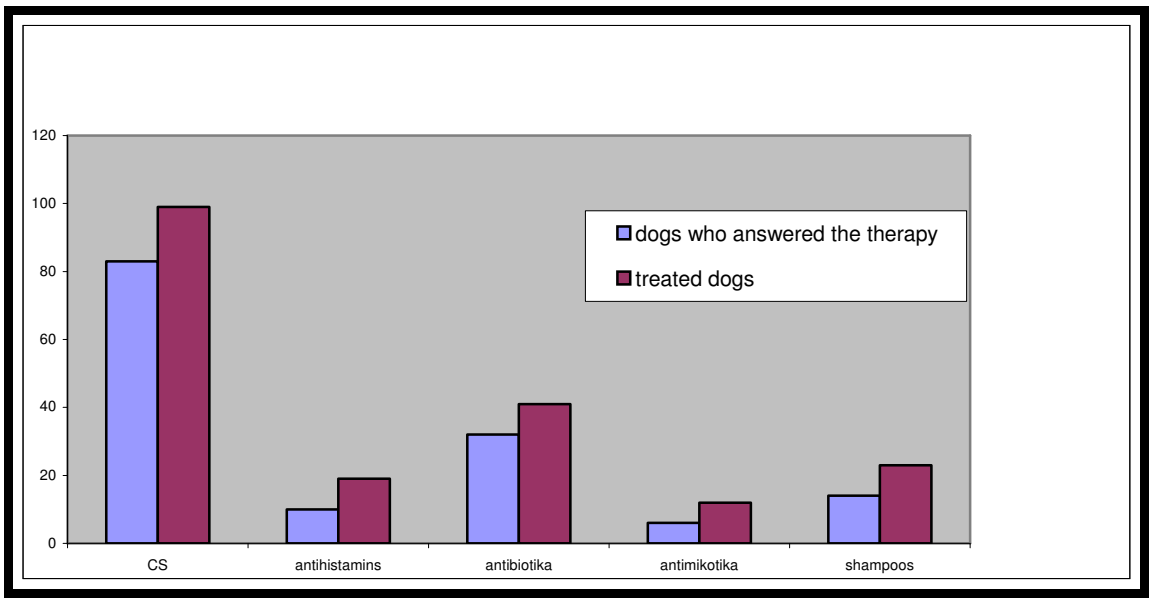

Figure 3. Pretreatments and their effects to clinical signs.

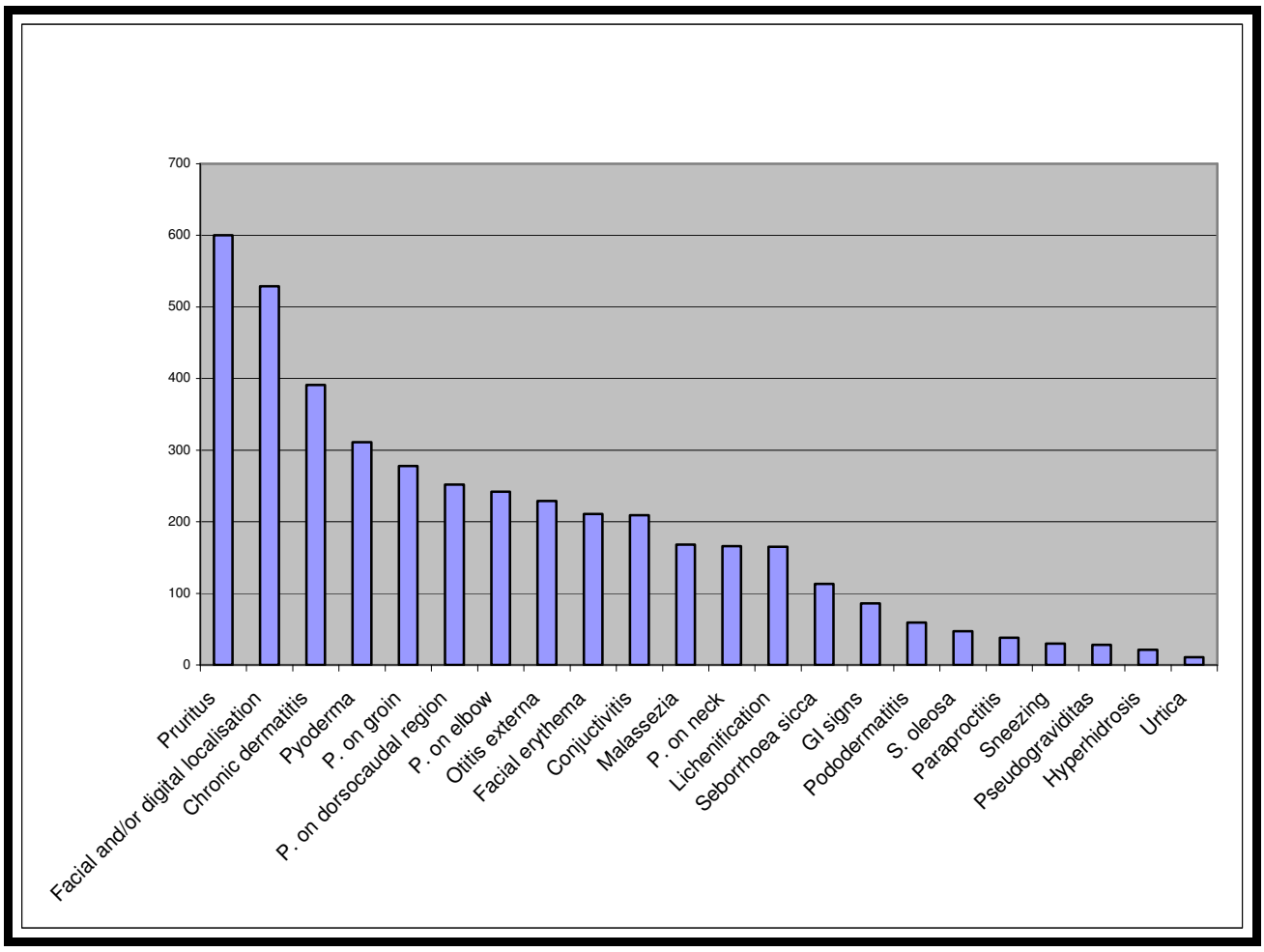

Figure 4. Prevalence of clinical symptoms.

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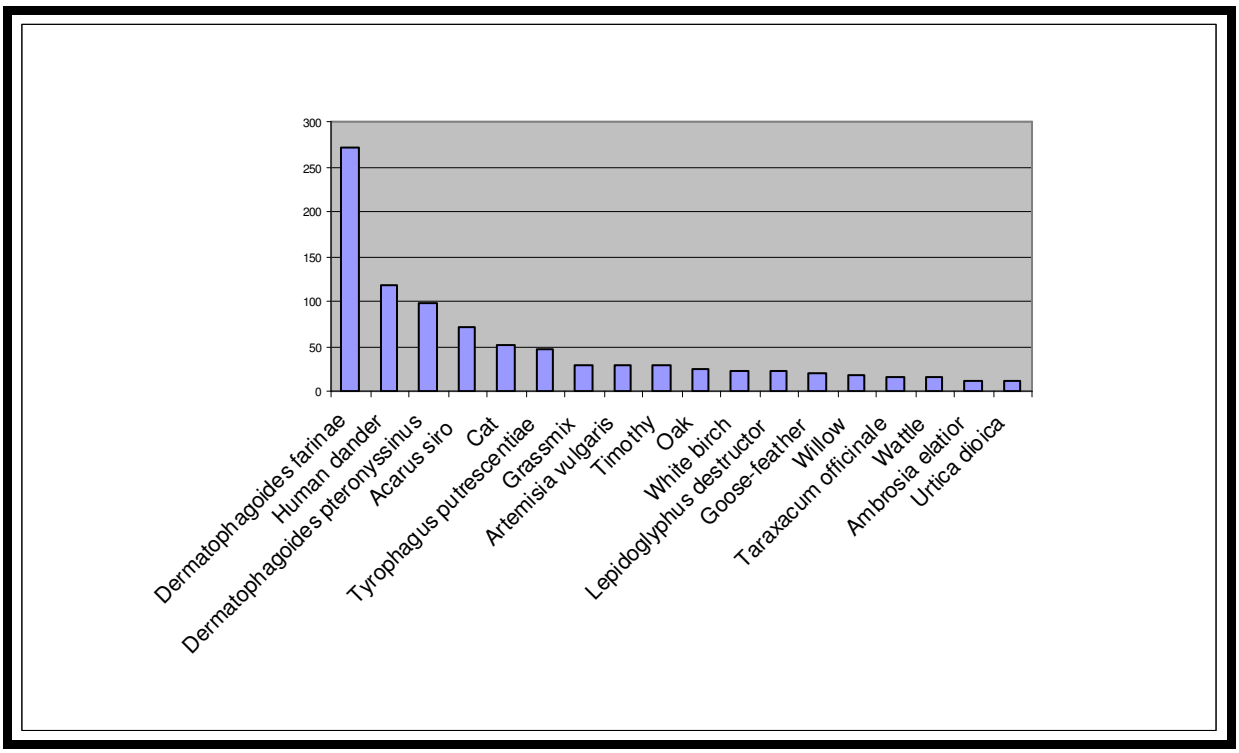

Figure 5. Positive reactions to allergens.

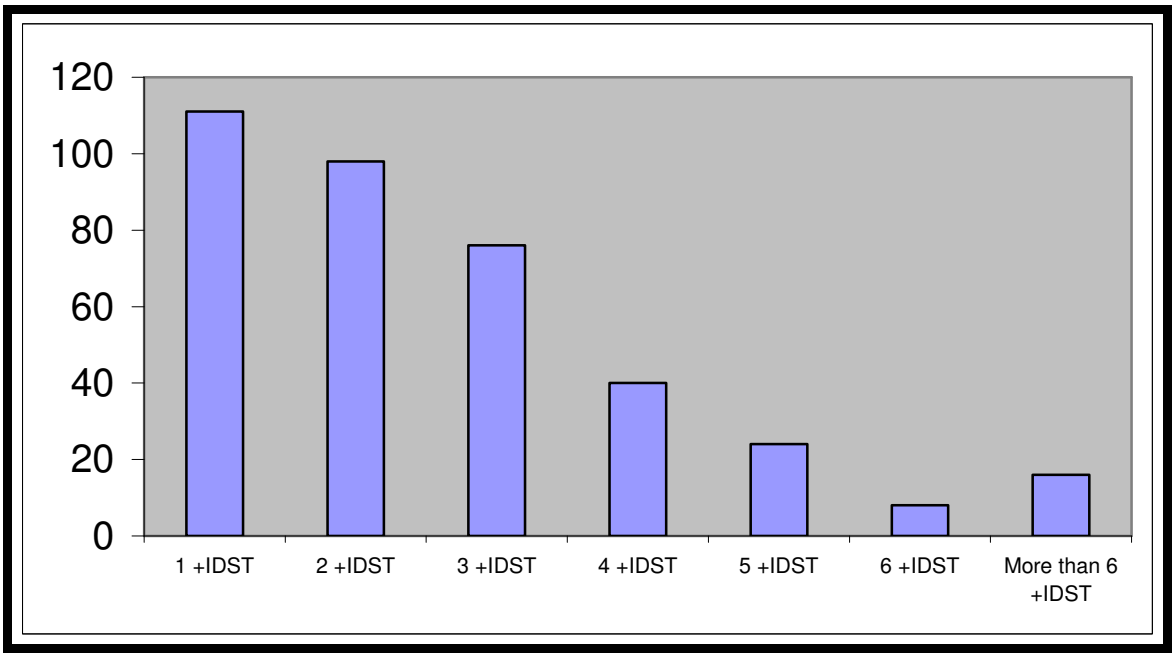

Figure 6. Number of positive reactions among the 373 positive patients.

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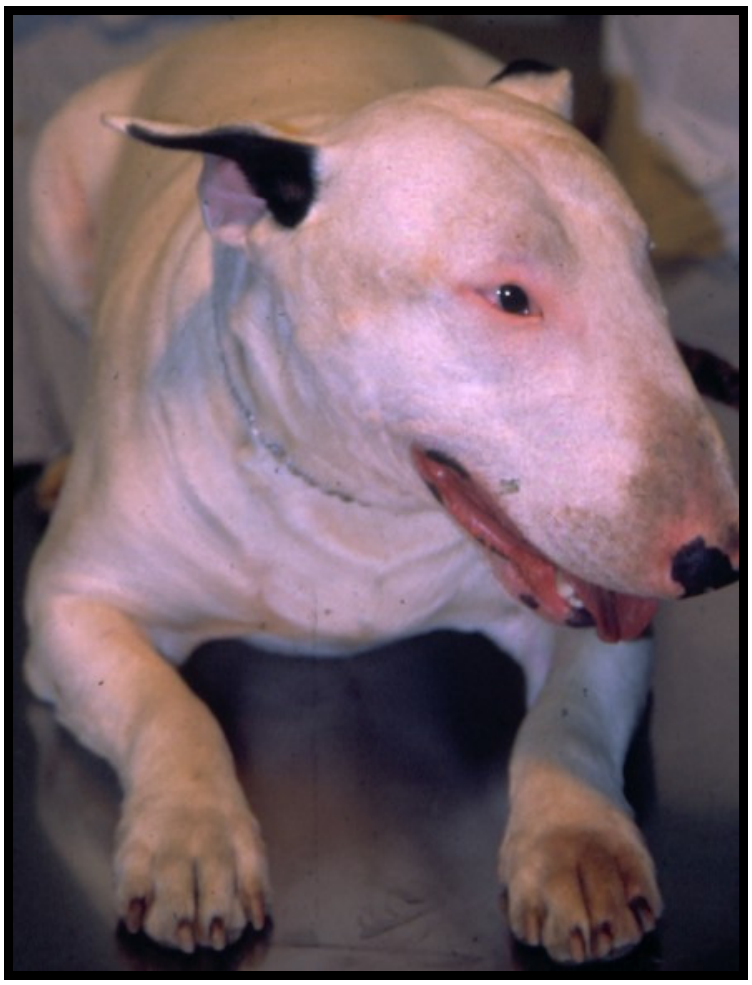

Figure A. Discoloration of the paws because of licking, and facial erythema of a bullterrier with atopic dermatitis.

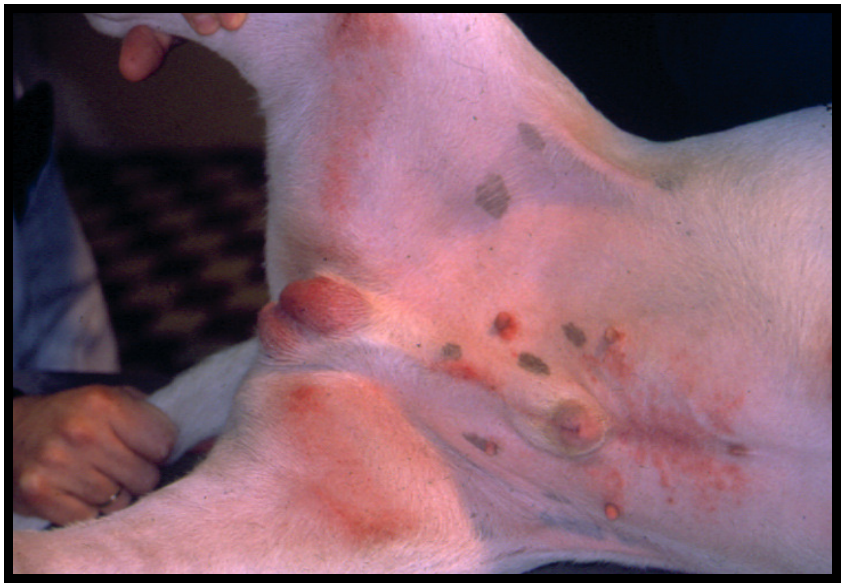

Figure B. Erythema on the lumbar region of a bullterrier with atopic dermatitis.

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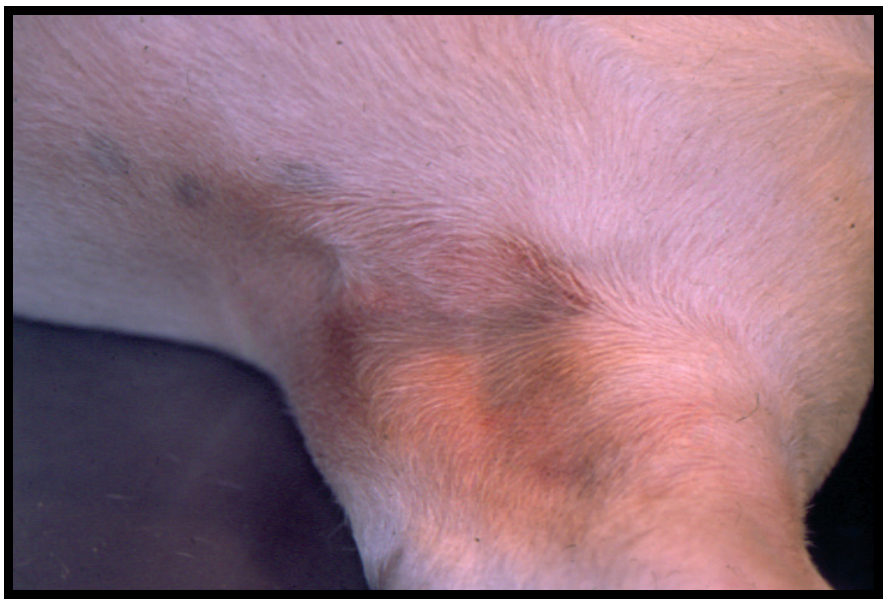

Figure C. Erythema on the axillar region of a bullterrier with atopic dermatitis.

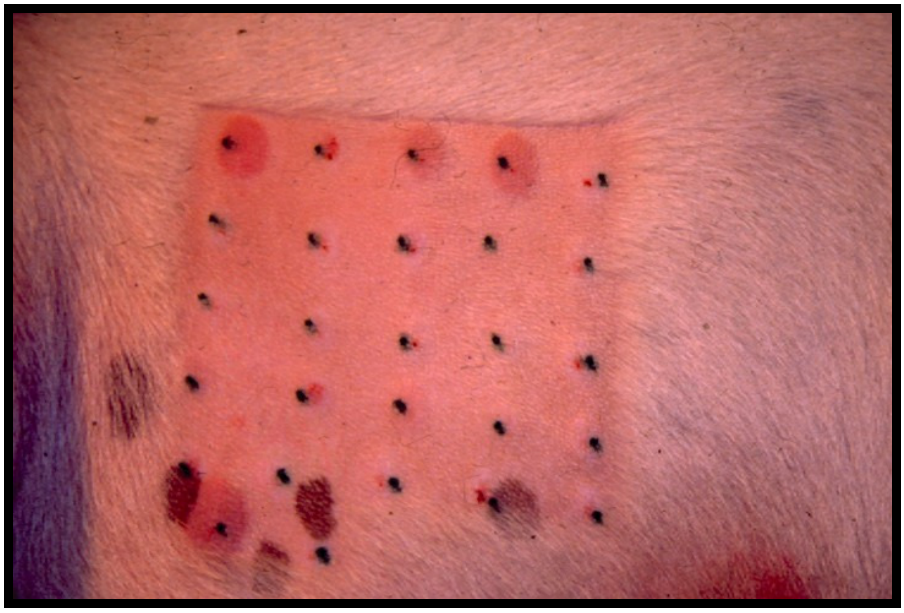

Figure D. Positive intradermal skin test reactions for three mites (Above: *D. farinae, A. siro, T. putrescentiae*, below: the positive control, histamine reaction) of a bullterrier with atopic dermatitis.

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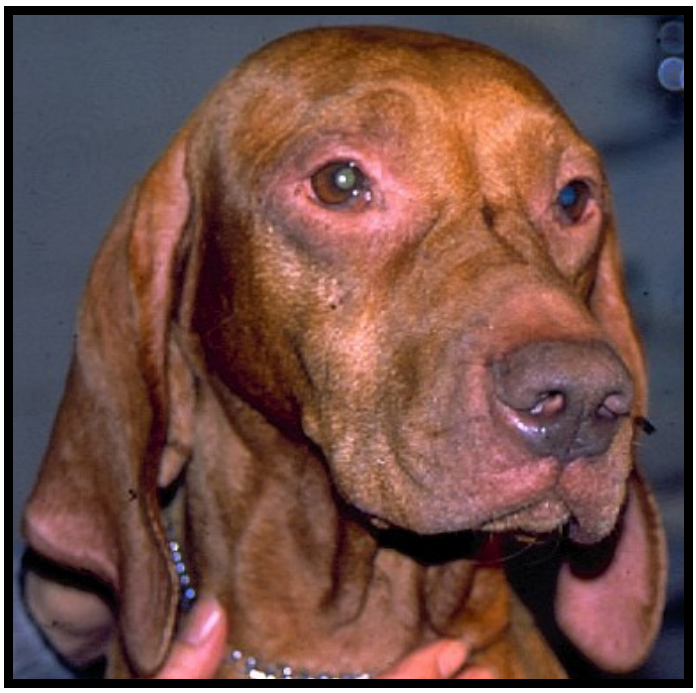

Figure E. Facial erythema, cheilitis and conjuctivitis in a Hungarian Vizsla with atopic dermatitis.

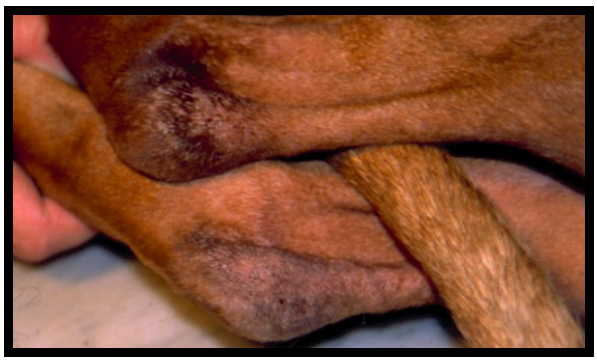

Figure F. Erythema and lichenification of the extensor surface of the carpal joint in a Hungarian Vizsla with atopic dermatitis.

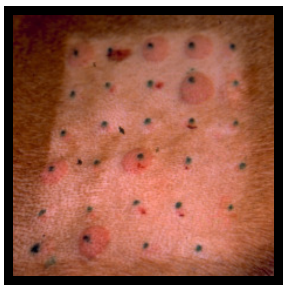

Figure G. Positive intradermal skin test reactions (for three mites: Above: *D. farinae, A. siro, T. putrescentiae*; human dander; *P. notatum, T. mentagrophytes*; below: the positive control, histamine reaction) of a Hungarian Vizsla with atopic dermatitis.

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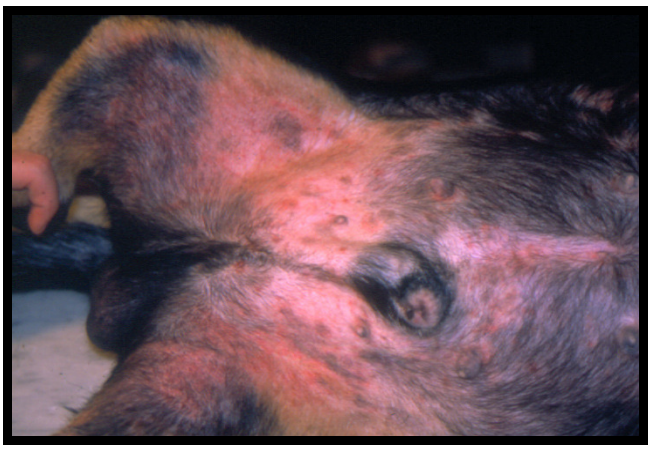

Figure H. Superficial pyoderma (erythema, papules, pustules, multifocal alopecia) on the lumbar region of a cross-breed dog with seasonal atopic dermatitis.

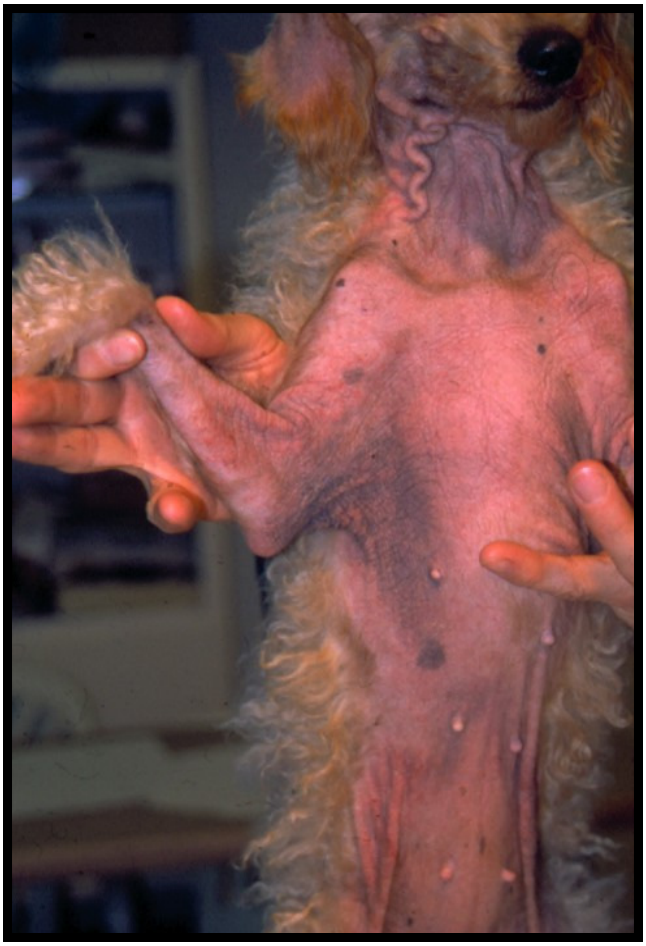

Figure I. Chronic aspecific dermatitis (traumatic alopecia, erythema, hyperpigmentation, lichenification) on the neck and the hole ventral trunk of a poodle with atopic dermatitis.

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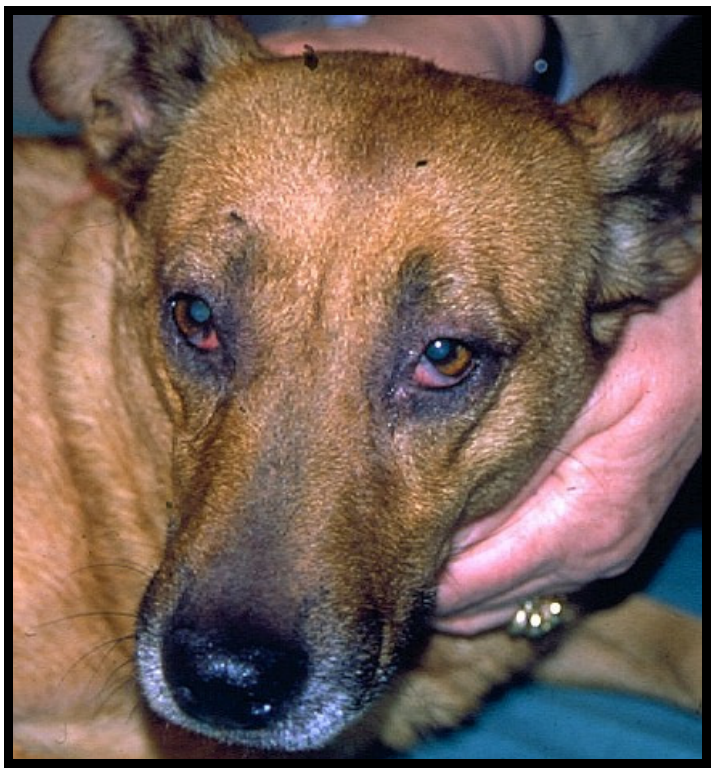


Figure J. Conjuctivitis, erythema, hyperpigmentation around the eyes of a cross-breed dog with atopic dermatitis.

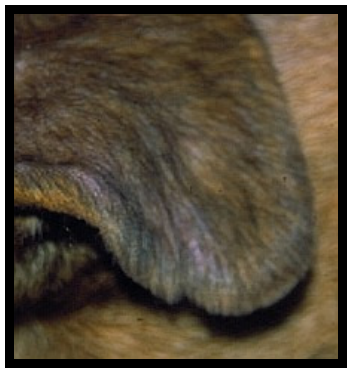


Figure K. Erythema, traumatic alopecia, excoriation on the ear pinnae of a cross-breed dog with atopic dermatitis.

Figure L. Pododermatitis of a cross-breed dog with atopic dermatitis.

First results of two newly developed ELISA methods for in vitro measurements of serum allergen-specific IgE in dogs

N. Tarpataki, O.Szabó, P.Vajdovich, M.Dörffler, G.Emődi, L.Papp, L.Vörös: First results of two newly developed ELISA methods for in vitro measurements of serum allergen-specific IgE in dogs

(Poster: 17th Annual Congress of the ESVD-ECVD Copenhagen, Denmark, 2001. 09. 27-29)

Tarpataki N. , Bagdi N. , Magdus M., Papp L., Vörös K., M. Dörffler, G. Emődi: Előzetes eredmények a kutyák intradermális allergiás bőrtesztjének és a vér allergén-specifikus IgE típusú ellenanyag-szintjének összehasonlításáról

(Akadémiai Beszámolók, Budapest, SZIE ÁOTK, 2000. 01. 28.)

Tarpataki N., Szabó O., Bagdi N., M. Dörffler: Kutyák intradermális allergiás bőrtesztjének és a vér allergén-specifikus IgE típusú ellenanyagszintjének összehasonlítása

(Akadémiai Beszámolók, Budapest, SZIE, ÁOTK, 2001. 01. 25.)

Introduction

The exact diagnosis for the causal treatment of canine AD is very important. The more sensitive serology tests are needed next to the "golden standard" intradermal skin test (IDT) so as to have a more exact diagnosis. The aim of this work was to use the newly developed two ELISA systems by INTEX (INTEX Pharmazeutica AG Hofackerstrasse 77 CH-4132 Muttenz Tel + 41 (0) 61 465 90 70 Fax + 41 (0) 61 465 90 71 (www.intex-diagnostika.com)) to measure the allergen-specific IgE in atopic dogs (Figure 1) **which were examined by IDT too and to compare the results of the three different diagnostic tests.**

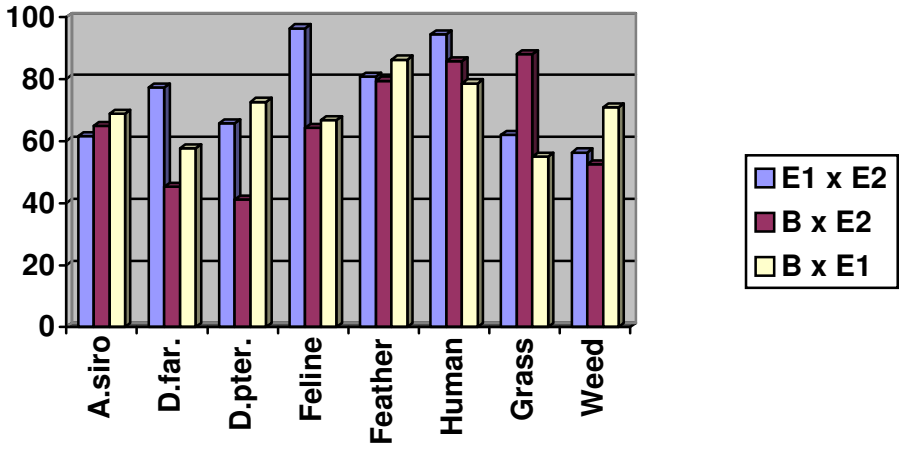
Materials and methods

The author of this work examined the correlation among in vivo (IDT) and two new *in vitro* tests applied in the diagnosis of canine atopic disease caused by inhaled allergens among 84 dogs of different breed and age presented at the dermatology consultation of our Department between 1st of September in 1999 and 16th of May in 2000. Patients were selected for the performance of supportive clinical examination on the basis of history and clinical symptoms (Willemse, 1986). The INTEX developed the serology tests: a membrane-strip or blister technology (solid phase ELISA, E1) and a microtiter plate technology (fluid phase ELISA, E2) to measure the allergen-specific IgE in dogs. The linkage between the IgE and the allergen linked to the solid phase or floating in the fluid phase can be seen by adding an anti-canine IgE labelled with peroxidase enzyme and a chromogenic substrate. The 23 ARTUVETRIN test allergens were used for IDT (**Figure 2**) but we analysed the results of only 8 allergens which were: *A. siro*, *D. farinae*, *D. pteronyssinus*, cat epithelium, feather mixture, human epithelium, grass pollen mixture, weed pollen mixture. The results of one *in vivo* and two new *in vitro* tests were assessed by each allergen and each method. A statistical program (SPSS Version 7.5) was used to analyse the results. The three

different tests were examined by pairs in respect of agreement and divergence of negative and positive results of each method; the sensitivity and specificity of the tests were assessed one by one.

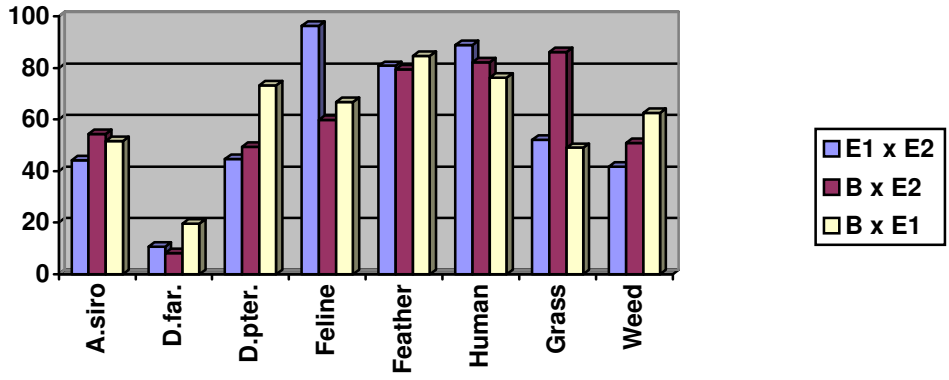
Results

The all results of all three methods corresponded with each other in 69,6%.

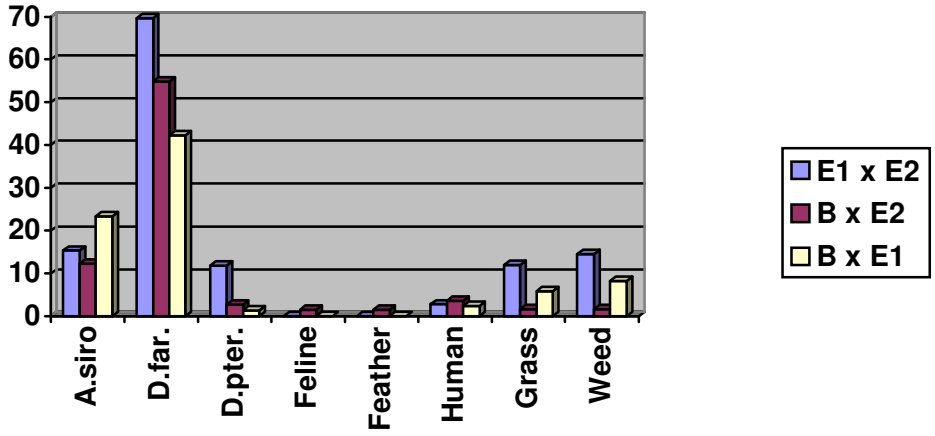


1. Figure **Agreement among the results of the three diagnostic methods, membrane strip ELISA (E1), microtiter plate assay (E2), and intradermal skin test (B) during the examination by pairs (%).**

Average agreement of the diagnostic methods was the highest between the two ELISA methods during the examination by pairs (74,3%), was lower between the intradermal skin test and membrane strip ELISA (69,5%), and was the lowest between the intradermal skin test and microtiter plate assay (65,1%). The average agreement of the diagnostic methods for each allergen was the highest with human epithelium, feather mixture and feline epithelium (Table 1)**. If we examine the results by each allergen and each diagnostic method** (Table 1, Figure 1)**, two ELISA methods for feline epithelium and human epithelium and intradermal skin test with microtiter plate assay for grass mixture agreed in the highest percentage. Agreement was the lowest at *D. pteronyssinus*, *D. farinae* house dust mites and weed mixture, all of them between intradermal skin test and microtiter plate assay** (Table 1)**. Agreement of the results by each allergen was the most uniform for feather mixture, *A. siro* flour mite and human epithelium** (Table 1)**. By each test matching this agreement the most uniform was between intradermal skin test and membrane strip ELISA** (Table 1)**. Agreement by each allergen was the most marginal with grass mixture *D. farinae* house dust mite, feline epithelium and *D. pteronyssinus* house dust mite; by each test matching the most marginal agreement was between intradermal skin test and microtiter plate assay** (Table 1)**.**



2/a. Figure **Agreement among the negative results of diagnostic methods, membrane strip ELISA (E1), microtiter plate assay (E2) and intradermal skin test (B) for each allergen during the examination by pairs (%).**

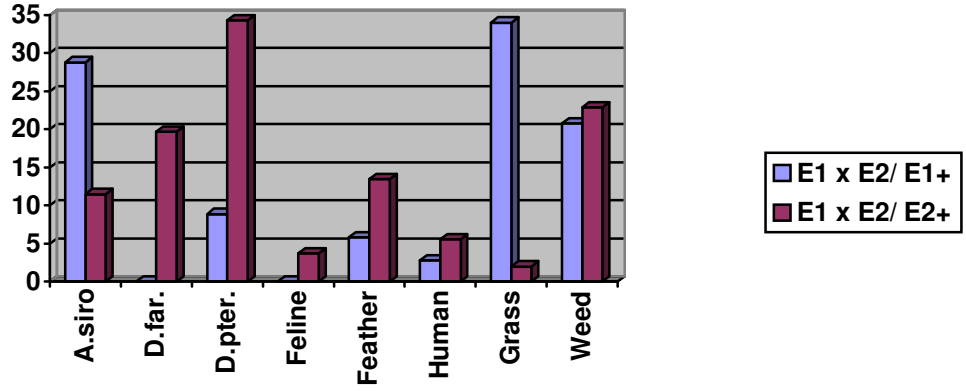


2/b Figure **Agreement among the positive results of the diagnostic methods: membrane strip ELISA (E1), microtiter plat assay (E2) and intradermal skin test (B) with each allergen during the examination by pairs (%)**

Since the two graphs complement each other (Figure 2/a-b)**, they will be assessed together. The positive results of tests examined by pairs** (Table 2, Figure 2/b) **agreed in the lowest percentage for feline epithelium, human epithelium and feather mixture at all three tests. The results of the two ELISA methods agreed in higher percentage for *D. farinae* house dust mite** (Table 2)**, grass mixture and weed mixture than the other matching test. The positive results of intradermal skin test and membrane strip ELISA agreed in some higher percentage for weed mixture and grass mixture than the results of intradermal skin test and microtiter plate assay** (Table 2)**. The positive results for *D. farinae* house dust mite agreed in extremely high percentage; this was the highest between the membrane strip ELISA and the microtiter plate assay, it was lower between intradermal skin test and microtiter plate assay** (Table 2)**. The positive results of intradermal skin test and membrane strip ELISA agreed in the lowest percentage for this allergen** (Table 2)**.**

In general it can be stated that negative results agreed in higher percentage than positive results. Agreement is remarkable between graphs showing negative results (Table 2, Figure 2/a) **and agreement of the results** (Table 1, Figure 1) **especially with feline epithelium, feather mixture, human epithelium, grass mixture and weed mixture. The high agreement of**

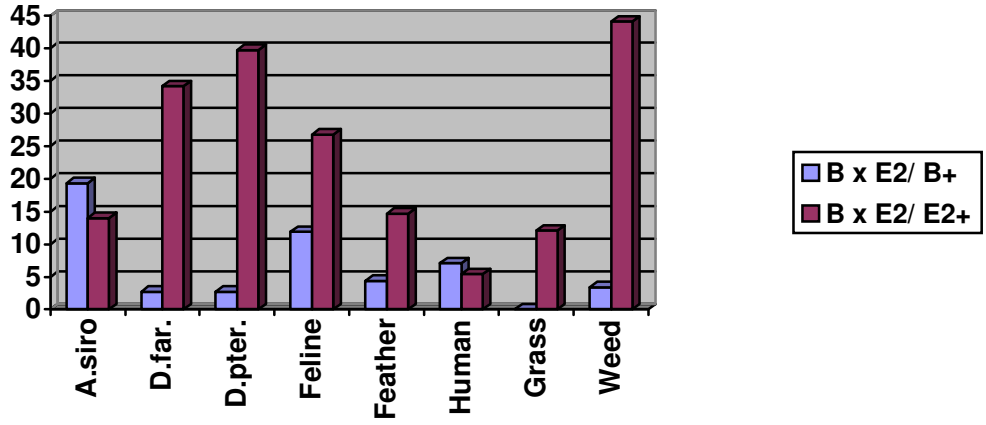
negative results for these allergens elevated the agreement of the test results (Table 1, Figure 1)**. For *D. farinae* house dust mite the curves of the two graphs were irregular, the numerous positive results agreed in high percentage in all tests** (Table 2)**, the very few negative reactions couldn't increase the agreement of negative results.**



3/a. Figure. **The ratio of positive results with the membrane strip ELISA (E1) and the microtiter plate assay (E2) when the other test pair presented negative results during the examination by**

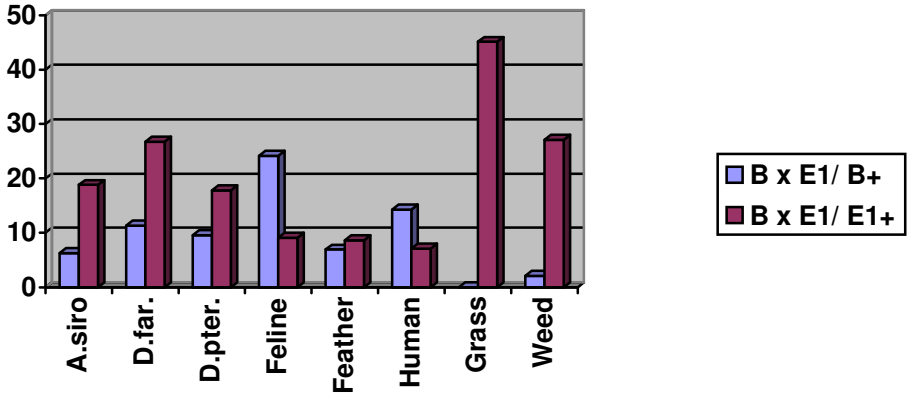
pairs (%)

In general it can be stated in this test the microtiter plate assay gave positive results in higher percentage than the membrane strip ELISA, except for *A. siro* flour mite and grass mixture (Table 3, Figure 3/a)**. In the latter cases membrane strip ELISA presented positive results more often than microtiter plate assay** (Table 3)**.**



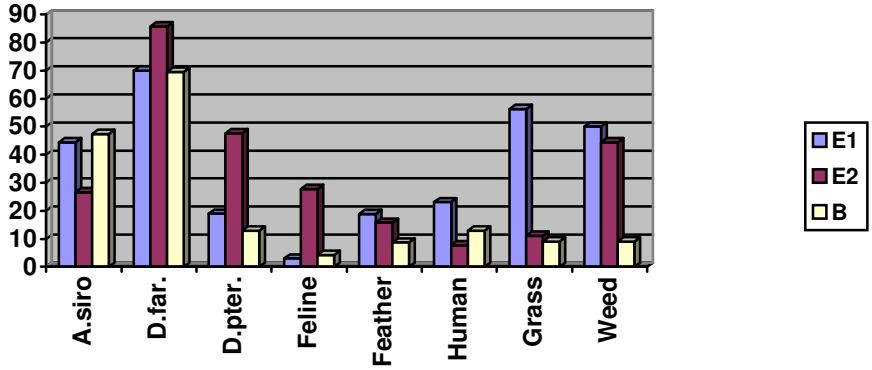
3/b. Figure**. The ratio of positive results of intradermal skin test (B) and microtiter plate assay (E2) when the other test pair presented negative results during the examination by pairs (%)**

By this graph (Table 3, Figure 3/b) **it can be stated in general that microtiter plate assay gave positive rations more often than intradermal skin test, except for human epithelium and *A. siro* flour mite allergens. Intradermal skin test showed positive reactions in higher percentage for these allergens** (Table 3)**. Microtiter plate assay presented more positive reactions than intradermal skin test in extremely high percent at *D. farinae*, *D pteronyssinus* house dust mites and weed mixture** (Table 3)**.**



3/c. Figure**. The ratio of positive results of intradermal skin test (B) and membrane strip ELISA (E1) when the other test pair presented negative results during the examination by pairs (%)**

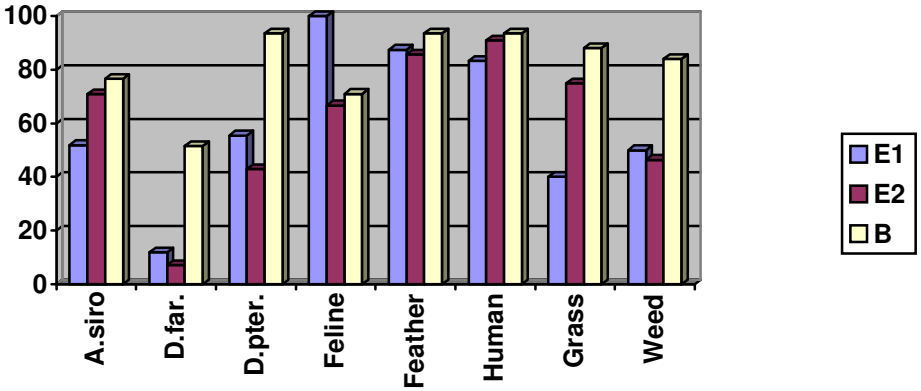
The membrane strip ELISA (Table 3, Figure. 3/c) **gave more positive results than the intradermal skin test, except for feline epithelium and human epithelium, where intradermal skin test showed positive reactions more often** (Table 3)**. The grass mixture and weed mixture membrane strip ELISA showed positive reactions in significantly higher percentage than the intradermal skin test** (Table 3)**. The feather mixture in both tests showed positive results in almost similar percentage** (Table 3) **when the other test pair gave negative results.**



4. Figure**. *Sensitivity* of the diagnostic methods: membrane strip ELISA (E1), microtiter plate assay (E2) and intradermal skin test (B)**

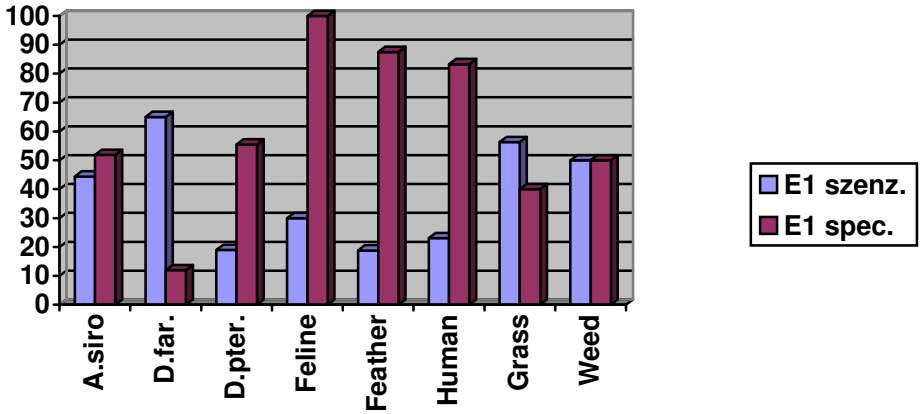
According to the average sensitivity values sensitivity of membrane strip ELISA was the highest, it was followed by microtiter plate assay (Table 4, Figure 4)**. Intradermal skin test seemed the least sensitive** (Table 4)**. Considering this graph sensitivity of all tests was high for**

***D. farinae* house dust mite** (Table 4)**. With the *A. siro* flour mite both the membrane strip ELISA and the intradermal skin test seemed sensitive, just like for at *D. pteronyssinus* house dust mite the microtiter plate assay. For the seasonal allergens (grass, weed mixture) sensitivity of the membrane strip ELISA was high** (Table 4)**. Sensitivity of each test was relatively low with feline epithelium, feather mixture and human epithelium** (Table 4)**.**



5. Figure. ***Specificity* of diagnostic methods, membrane strip ELISA (E1), microtiter plate assay (E2), intradermal skin test (B) by each allergen (%)**

Average specificity values (Table 5, Figure 5) **of intradermal skin test was the highest among all diagnostic methods, average specificity values of two ELISA methods were almost similar. With the feather mixture and human epithelium specificity of all three diagnostic methods was high** (Table 5)**. The two ELISA methods showed the lowest sensitivity for *D. farinae* house dust mite** (Table 4)**. Intradermal skin test presented the highest sensitivity for *D. pteronyssinus* house dust mite, grass mixture and weed mixture** (Table 4)**. For feline epithelium, specificity of the membrane strip ELISA seemed the highest** (Table 5)**. Specificity of two ELISA methods differed significantly for grass mixture and feline epithelium** (Table 5)**, but according to the above mentioned the latter allergen should be judged with reservations.**

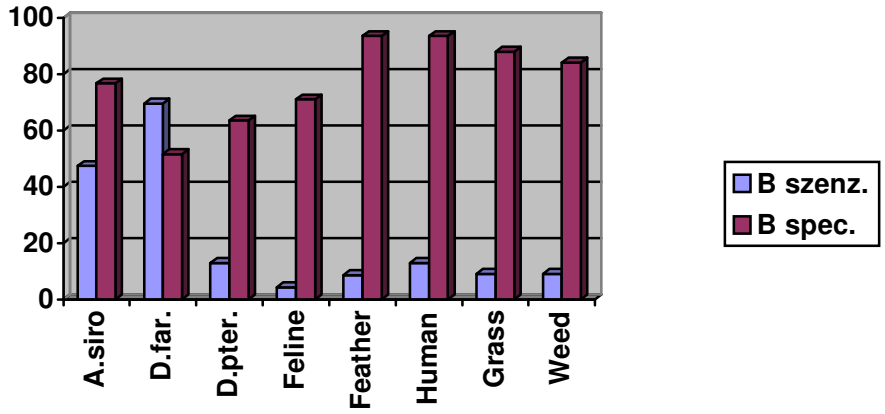


6/a.] **or each**

ral than specificity, except for D. farinae house dust mite and grass mixture, where sensitivity was higher (Table 4-5)**. For weed mixture sensitivity and specificity were similar** (Table 4-5)**.**

6/b. Figure. **Comparison of sensitivity and specificity of microtiter plate assay (E2) for each allergen (%)**

The specificity of the microtiter plate assay its specificity (Table 4-5, Figure 6/b) **was higher than its sensitivity, except for *D. farinae* house dust mite, where sensitivity was higher** (Table 4-5)**. For weed mixture and *D. pteronyssinus* house dust mite specificity and sensitivity were almost similar** (Table 4-5)**.**



6/c. Figure. **Comparison of sensitivity and specificity of intradermal skin test (B) for each allergen(%).**

Specificity of intradermal skin test (Table 4-5, Figure 6/c) **was higher in general compared to its sensitivity, except for *D. farinae* house dust mite, where sensitivity was higher.**

Table 1. **Agreement among the results of the three diagnostic methods, membrane strip ELISA (E1), microtiter plate assay (E2), and intradermal skin test (B) during the examination by pairs (%).**

	E1 X E2	B X E2	B X E1	Average
A. siro	61,5	64,9	68,8	65,1
D. farinae	77,3	45,2	57,7	60,1
D. pteronyssinus	65,7	41,1	72,6	59,8
Feline epithelium	96,3	64,2	66,7	75,7
Feather mixture	80,7	79,4	86,2	82,1
Human epithelium	94,4	85,7	78,6	86,2
Grass mixture	62,0	87,9	54,9	68,3
Weed mixture	56,3	52,5	70,8	59,9
Average agreement	74,3	65,1	69,5	

Table 2. **Agreement among the negative (-, +/-) and positive (+, ++, +++, ++++) results of the diagnostic methods, membrane strip ELISA (E1), microtiter plate assay (E2) and intradermal skin test (B) with each allergen during the examination by pairs (%).**

	E1	E2	B	E2	B	E1
	-	+	-	+	-	+
A. siro	44,2	15,4	54,4	12,3	51,6	23,4
D. farinae	10,6	69,7	8,2	54,8	19,7	42,3
D. pteronyssinus	44,7	11,9	49,3	2,7	73,2	1,4
Feline epithelium	96,3	0	59,7	1,5	66,7	0
Feather mixture	80,76	0	79,4	1,5	84,5	0
Human epithelium	88,9	2,8	82,1	3,6	76,2	2,4
Grass mixture	52,0	12,0	86,2	1,7	49,0	5,9
Weed mixture	41,7	14,6	50,8	1,7	62,5	8,3

Table 3. **The ratio of positive results of membrane strip ELISA (E1), microtiter plate assay (E2) and intradermal skin test (B) when the pairs in test matching presented negative results during the examination by pairs (%).**

	E1	**E2**	**B**	**E2**	**B**	**E1**
A. siro	**28,8**	**11,5**	**19,3**	**14,0**	**6,3**	**18,8**
D. farinae	**0**	**19,7**	**2,7**	**34,2**	**11,3**	**26,8**
D. pteronyssinus	**8,9**	**34,3**	**2,7**	**39,7**	**9,6**	**17,8**
Feline epithelium	**0**	**3,7**	**11,9**	**26,8**	**24,2**	**9,1**
Feather mixture	**5,8**	**13,5**	**4,4**	**14,7**	**6,9**	**8,6**
Human epithelium	**2,8**	**5,6**	**7,1**	**5,4**	**14,3**	**7,1**
Grass mixture	**34,0**	**2,0**	**0**	**12,1**	**0**	**45,1**
Weed mixture	**20,8**	**22,9**	**3,4**	**44,1**	**2,1**	**27,1**

Table 4. ***Sensitivity*** **of diagnostic methods (%).**

	E1	E2	B
A. siro	44,4	26,7	47,4
D. farinae	70,0	85,7	69,6
D. pteronyss.	19,0	47,6	13,0
Feline epith.	30,0	27,8	4,3
Feather mix.	18,8	15,8	8,7
Human epith.	23,1	7,7	13,0
Grass mix.	56,3	11,1	9,1
Weed mix.	50,0	44,4	9,1
Average sens.	38,3	33,4	21,8

Table 5. ***Specificity*** **of diagnostic methods (%).**

	E1	E2	B
A. siro	51,9	70,8	76,7
D. farinae	12,0	7,1	51,6
D. pteronyss.	55,5	42,9	93,5
Feline epith.	100,0	66,7	71,0
Feather mix.	87,5	85,7	93,5
Human epith.	83,3	90,9	93,5
Grass mix.	40,0	75,0	88,0
Weed mix.	50,0	46,4	84,0
Average spec.	60,0	60,7	81,5

Conclusions

The authors examined three different methods for identifying allergens causing atopic dermatitis in dogs. The results have been evaluated by allergens and by methods. The results of three different methods and of 8 different allergens were in agreement in 69,9% of the cases. Of course this is an average number and the difference among allergens is high. The agreement of our results in case of grass-pollens (68,2%), weed-pollens (59,8%), and house dust mites (60%) is higher than what is stated in the literature (Kleinbeck et al. 1989; Codner and Griffin 1966). The highest agreements in our measurements were in case of human epithelium (86,2%), feather mixture (82,1%) and cat epithelium (75,7%). Of course correlation of the negative results was higher because of the higher number of negative results except the *D. farinae* house dust mite, which allergen is the most recurrent allergen. This is corresponding with the literature (Codner and Griffin 1996). The sensitivity of all tests was the highest in case of *D. farinae* house dust mite (E1: 70%, E2: 85,7%, IDT: 69,6%). The sensitivity of ELISA tests was much more higher in case of seasonal allergens (grass-pollens: E1: 56,3%; weeds-pollens: E1: 50%, E2: 44,4%, IDT: 9,1%). The reason for this difference could be, that the IgE antibodies in the blood can be detected while the IgE molecules connected to the mastocytes in the skin are reduced. The highest specificity showed the IDT (81,5%), then the E2 (60,5%) and the E1 (60,0%) come in due course. This corresponds to the tendency published in the literature (Codner and Griffin 1996). The comparison of the two types of ELISA was new in this work, there's no available data about this in the literature, so far.

These two ELISA methods are under further development mainly to improve the sensitivity and specificity with different scale by applied methods and by the allergens.

The described methods have important roll in the diagnosis of canine atopy. Because of the difference between false positive and false negative contingencies of the methods it would be ideal to performe each method to identify the causal allergens in the atopic dogs. If a veterinary surgeon knows the specificity and sensitivity of theses tests he can "use" the results of the different diagnostic methods for the treatment.

Figure 1. INTEX test strips, Allergoset Canine for indoor, and outdoor allergens. (www.intex-diagnostika.com)

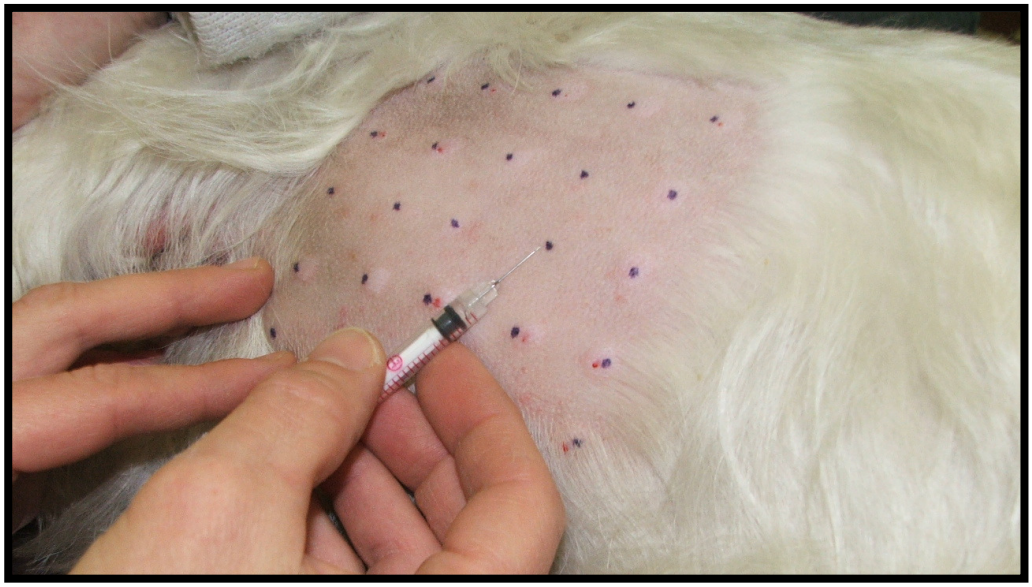


Figure 2. Intradermal application of allergens.

5. Chapter IV

Evaluation and innovation of an enzyme-linked immunosorbent assay (ELISA) and the intradermal skin tests in 231 dogs

Noémi Tarpataki, Beat Bigler, Péter Vajdovich, Károly Vörös: Evaluation of an enzyme-linked immunosorbent assay (ELISA) 162 dogs. Submitted to *Journal of Small Animal Practice*

Introduction:

In Hungary the intradermal skin testing has been used to identify the allergens in canine atopic dermatitis for over 10 years. The serological tests are available only for last years. That is why it is important to know in the small animal practice which test to choose and how to evaluate the results. The aim of this study was to develope, to increase the sensitivity of allergen-specific IgE test (imovet biocheck: Labor Laupeneck/imovet bg, Laupenstrasse 33, CH-3008 Berne, Switzerland, www.laupeneck.ch) (**Figure 1**) and to evaluate the correlation of ELISA measuring serum allergen-specific IgE test (imovet biocheck) and intradermal skin testing (Artuvetrin) in canine atopic dermatitis and to be comparable with the other allergen-specific-IgE-ELISA-evaluating studies. Regarding that each study counted with different values all of them were calculated in this study. Occurance of the different allergens in Hungary, specificity, sensitivity, positive predective value, negative predective value, accuracy, positive, negative and total correlation, agreement of positive and negative results in two ways, significance of correlation were evaluated too.

Materials and methods

After recording a detailed history from the owner, clinical dermatological consultations and examinations; blood sera were collected and sent in two steps (m_1=69; m_2=162) for measurement of allergen-specific IgE using imovet biocheck. Intradermal skin tests were also performed on 212 (62+150) pruritic dogs with the clinical diagnosis of atopic dermatitis and 19 (7+12) on clinically healthy dogs. All of them were admitted to our Department between September 2002 and 2005 December. Prior to skin testing all anti-inflammatory drug therapy, including oral and topical glucocorticoids and anti-histamines, were discontinued for at least 3 weeks and 10 days, respectively. Skin skrapings were taken from all the pruritic dogs, and if pyoderma or fungi/yeast was present clinically and in the bacterial and fungal culture the patient was treated by the appropiate antibiotic or antimicotic drug for a minimum of 3 weeks before sampling. For excluding the *Sarcoptes*-infestation scabicidal therapy (selamectin (Stronghold® spot on: Pfizer Inc. Animal Health (Plant) 1107 S. State Route, 291 Lees Summit, MO, USA) as spot on solution for systemic treatment (twice in 4 weeks interval)), was prescribed in all (212) patients. All patients (212) had to have an 8 week elimination monodiet to exclude the adverse food reaction, 200 dogs had had it before the examination and 12 patients had it after the examination. The elimination monodiet was recommended to contain home-cooked one sort of protein (lamb,

fish, rabbit, horse, venison, deer etc.) which has never been eaten by the dog and one sort of carbohydrate (potato or rice) boiled in salty water. Only apple and carrot were allowed to give next to the monodiet.

Blood samples were taken from all dogs using BD Vacutainer™ SST (serum sample tube) (**Figure 2**). After sampling, the sera were separated, immediately frozen and stored at - 17 °C until sending to the Labor Laupeneck for measurement of IgE using imovet biocheck. The blood samples were measured in two groups. Firstly 69 sera were measured, then after further collection other 162 sera were sent for evaluation. The Labor Laupeneck increased the sensitivity of the serological test in the meantime. They were then testing allergen-specific IgE, specific for house dust mites (*D. farinae*, *D. pteronyssinus*) meal mite (*A. siro*), copra mite (*T. putrescentiae*), hay mite (*L. destructor*), grassmix, ragweed (*Ambrosia elatior,* A.e.), stinging nettle (*Urtica dioica*, U.d.), plantain (*Plantago lanceolata*, P.l.), mugwort (*Artemisia vulgaris*, A.v.), birch (*Betula pendula*, B.p.)/ alder (*Alnus glutinosa*, A.g.)/ hazel (*Corylus avellana*, C.a.) mixture and flea-allergen. The sera were analyzed using indirect ELISA (Imovet Biocheck) containing the D9 monoclonal antidbody against canine IgE. The allergen set for intradermal skin testing (Artuvetrin Test solutions) contained the same allergens except for the *alder*. Only single allergens were used for skin testing, the tested grasspollens were orchard grass (*Dactylis glomerata*, D.g.), timothy (*Phelum pratense*, Ph.p.) and bleu grass (*Poa pratensis*, P.p.).

As **positive control group** for atopic dermatitis we considered (n_1=50 and n_2=150) 200 dogs of the 212 pruritic patients. The diagnosis of atopic dermatitis relied upon a careful evaluation of the owner's history and presence of appropriate clinical signs. 3 major and 3 minor criteria (out of the IDT and ELISA tests) of Willemse were established in these dogs. Pyoderma, *Malassezia* infection and parasite infestations (e.g. fleas and sarcoptes mites) were excluded by clinical and additional examinations and/or by the appropiate treatments. In these 200 dogs the 8 week long elimination monodiet was not succesful. Positive test reactions in this group were considered as true positive reactions (TP) and negative results as false negative (FN).

The other 12 pruritic dogs were on an 8 week long elimination monodiet only after the blood sampling and intradermal skin testing. 3 major and 3 minor criteria (out of the IDT and ELISA tests) of Willemse were established in these dogs, too. These patients were pruritic, **non-atopic dogs but positive for adverse food reaction (AFR)**. The positive test reactions of this group were considered as false positive (FP) reactions and the negative test results of this dogs were considered as true negative (TN) results.

The **negative control group** consisted of 19 (7+12) dogs which arrived at the Szent István University for their annual vaccinations showing no clinical signs of pruritus or any type of skin lesions for a minimum of 2 months. They were tested by the IDT and their sera were tested for

allergen-specific IgE, too. The positive test reactions of the negative control group were considered as false positive (FP) reactions and the negative test results as true negative (TN) reactions.

During evaluation of the tests the negative control dogs (7+12) and non-atopic dogs with AFR (12) were considered as negative control dogs for atopic dermatitis (k_1=7+12=19 and k_2=12). (The list of abbreviation is the following:)

All the results are found in the **Tables 1-9**, the first results (m_1=69) are in tables "A", and the results of the second group can be found in Tables "B".

The prevalence/proportion of the positive test reactions by allergens (See **Table** 1) in the positive control group and overall (**Table 2/A-B, 3/A-B**) was counted.

Table 1. Allergens included in both (ELISA and IDT) test panel; and proportion of dogs that tested positive for each allergen, for both test (number and per cent) in atopic dogs (n_1=50 in the first row; n_2=150 in the second row).

Allergen	ELISA proportion positive	ELISA proportion positive (%)	IDT proportion positive	IDT proportion positive (%)
Dermatophagoides farinae	24 88	48 59	31 79	62 53
Dermatophagoides pteronyssinus	15 10	30 7	15 4	30 3
Acarus siro	19 86	38 57	15 74	30 49
Tyrophagus putrescentiae	10 109	20 73	3 72	6 48
Lepidoglyphus destructor	2 35	4 23	5 34	10 23
Grassmix	3 22	6 15	2 10	4 7
Ambrosia elatior (ragweed)	9 33	18 22	9 43	18 29
Urtica dioica (stinging nettle)	0 1	0 1	0 7	0 5
Plantago lanceolata Plantain	1 16	2 11	3 5	6 3
Artemisia vulgaris (mugwort)	5 36	10 24	7 28	14 19
Betula pendula/Alnus glutinosa/Corylus avellana (birch/alder/hazel)	8 18	16 12	9 5	18 3
Flea	2 21	4 14	3 19	6 13

The overall specificity and sensitivity (**Table 2/A-B, 3/A-B**) of the diagnostic methods was calculated as follows:

Specificity (Sp)(%)= TN/ (TN+FP)

Sensitivity (Sn)(%)= TP/ (TP+FN)

The average sensitivity and specificity of both test methods (**Table 5/A-B**) were calculated too.

The positive, negative predective values and accuracy were calculated for both tests overall as follows (**Table 2/A-B, 3/A-B**):

positive predective value (PPV) (%) =TP/(TP+FP)

negative predective value (NPV) (%) =TN/ (TN+FN)

Accuracy (Acc) (%) = (TP+TN)/ total (m)

Positive, negative and total correlation overall, by allergens and average were counted as follows (**Table 4/A-B**):

positive correlation (PC) (%) =number of common positive test results/number of positive control group= (ELISA+IDT+)/ n

negative correlation (NC) (%) = number of common negative test results/number of positive control group= (ELISA-IDT-)/ n

total correlation (TC) (%) = sum of the positive and negative correlation =PC+NC

To calculate sensitivity for each allergen and each test method, the sensitivity for ELISA was determined using positive IDT results as positive control group and inversely, the sensitivity for intradermal skin test was determined using the serum positive test results as positive control group (**Table 5/A-B**).

Sn(ELISA)(by allergens)= (ELISA+IDT+)/((ELISA+IDT+)+(ELISA-IDT+))

Sn(IDT)(by allergens)=(ELISA+IDT+)/((ELISA+IDT+)+(ELISA+IDT-))

To count specificity for each allergen and each test method, the specificity for both tests was determined by any false positive reactions in the negative control group (k_1=19; k_2=12).

Sp(ELISA)= ELISA+/k

Sp(IDT)= IDT+/k

Average of the specificities and sensitivities were also calculated.

Agreements between IDT and ELISA test for positive results were determined overall and for individual allergens. The agreements of the positive results (Apos) and negative results (Aneg) were counted in two ways by each allergen as follows (**Table 6/A-B**):

Apos= the number of the common positive results of both test methods / the number of the results when both or one of the tests gave positive reaction=

=(ELISA+IDT+)/((ELISA+IDT+)+(ELISA+IDT-)+ (ELISA-IDT+))

Apos (Codner)= (ELISA+IDT+)/((ELISA+IDT+)+(ELISA-IDT+))= Sn(ELISA)(by allergens)

Aneg= the number of the common negative results of both test method /the number of the results when both or one of the tests gave negative reaction=

=(ELISA-IDT-)/((ELISA-IDT-)+(ELISA-IDT+)+(ELISA+IDT-))

Aneg(Codner)= (ELISA-IDT-)/((ELISA-IDT-)+(ELISA+IDT-)))=Sp+ ELISA

(Sp+ is the specificity of ELISA counted in the positive control group by allergens (not in the negative control group!))

Correlation between results of different tests for reactions to each allergen were assessed using two-tailed Pearson bivariate correlation/ Pearson's correlation coefficient and Kappa value too. The differences in test results between IDT and serological tests in individual dogs were compared using McNemar's matched chi-squared test (**Table 7/A,B**):

All the calculations were done by SPSS Version 8.0 for Windows.

Results

Patients with adverse food reaction:

This group was composed of 7 females, 5 males, the ages of these 12 dogs were between 1 and 4 years. These patients were pruritic, 3 major and 3 minor criteria (out of the IDT and ELISA tests) of Willemse were established at the clinical examination. In all cases both the IDT and the allergy-serology were negative so no cause of the allergic dermatitis could have been proven after the examinations. But the elimination monodiet and the provocation diet which was performed after the clinical examination were positive in all these patient. There was not any false positive reaction but 12 true negative results for the tests in this group (**Table 2/A, 3/A**). These 12 dogs were considered as negative control patients for atopic dermatitis so there were (k_1=12+7=) 19 negative control dogs in the first measurement.

Negative control groups:

These groups were composed of 10 females and 9 males, the ages of these 19 dogs were between 1 and 6 years.

One dog of the first group (k_1=19) gave positive reactions both in the IDT and the allergen-specific IgE serology for *D. farinae*. This dog had positive test results by ELISA test for *A. siro* and *T. putrescentiae* too. Therefore the **specificity of ELISA for *D. farinae, A. siro*** and ***T. putrescentiae*** and **the specificity of IDT for *D. farinae* were 94.47%**. The **specificity** of both tests for all the **other allergens was 100%** (**Table 5/A-B**). The overall specificity of ELISA and IDT were 94.47% and 94.47%, the average specificity of ELISA and IDT were 98.82% and 99.61% (**Table 2/A, 3/A**).

Table 2/A. Cross–tabulation and results of ELISA test with respect to clinical diagnosis among all dogs (m_1=69) (AFR+:dogs with adverse food reaction, Sp=specificity, Sn=sensitivity, PPV=positive predective value, NPV=negative predective value, Acc=accuracy, TP=true positive, TN=true negative, FP=false positive, FN=false negative).

m_1=69	Clinical	diagnosis	for	atopic	dermatitis				
ELISA	Positive	Negative	Negative (AFR+)	Total	Sp	Sn	PPV	NPV	Acc
Positive	30,00(TP)	1,00(FP)	0(TP)	31,00					
Negative	20,00(FN)	6,00(TN)	12(TN)	38,00					
Total	50,00	7,00	12,00	69,00	94.47%	60%	96.77%	47.37%	69.57%

Table 2/B. Cross–tabulation and results of ELISA test with respect to clinical diagnosis among all dogs (m_2=162) (AFR+:dogs with adverse food reaction, Sp=specificity, Sn=sensitivity, PPV=positive predective value, NPV=negative predective value, Acc=accuracy, TP=true positive, TN=true negative, FP=false positive, FN=false negative).

m_2=162	Clinical	diagnosis	for	atopic	dermatitis				
ELISA	Positive	Negative	Negative (AFR+)	Total	Sp	Sn	PPV	NPV	Acc
Positive	132(TP)	0(FP)	0(TP)	132					
Negative	18(FN)	12(TN)	0(TN)	30					
Total	150	12	0	162	100%	88%	100%	40%	89%

Table 3/A. Cross–tabulation and results of IDT with respect to clinical diagnosis among all dogs (m_1=69) (AFR+:dogs with adverse food reaction, Sp=specificity, Sn=sensitivity, PPV=positive predective value, NPV=negative predective value, Acc=accuracy, TP=true positive, TN=true negative, FP=false positive, FN=false negative).

m_1=69	Clinical	diagnosis	for	atopic	dermatitis				
IDT	Positive	Negative	Negative (AFR+)	Total	Sp	Sn	PPV	NPV	Acc
Positive	40(TP)	1,00(FP)	0(TP)	41,00					
Negative	10(FN)	6,00(TN)	12(TN)	28,00					
Total	50,00	7,00	12,00	69,00	94.47%	80%	97.56%	64.29%	84.06%

Table 3/B. Cross–tabulation and results of IDT with respect to clinical diagnosis among all dogs (m_2=162) (AFR+:dogs with adverse food reaction, Sp=specificity, Sn=sensitivity, PPV=positive predective value, NPV=negative predective value, Acc=accuracy, TP=true positive, TN=true negative, FP=false positive, FN=false negative).

M_2=162	Clinical	diagnosis	for	atopic	dermatitis				
IDT	Positive	Negative	Negative (AFR+)	Total	Sp	Sn	PPV	NPV	Acc
Positive	110(TP)	0(FP)	0(TP)	110					
Negative	40(FN)	12(TN)	0(TN)	52					
Total	150	12	0	162	100%	73%	100%	23%	75%

In the second group (k_2=12) the specificity of both tests for all allergens was 100% (**Table 5/B**). The overall specificity of ELISA and IDT were 100-100% (**Table 2 /B, 3/B**) the average specificity of ELISA and IDT were 100-100% in the second group, too. (**Table 5 /B**).

Positive control groups

The group was composed of 63 females, 81 males and 6 spayed females, the ages of these (n_1=50; n_2=150) 200 dogs were between 1 and 10 years in this group. The most common breeds presented were German sheperd dog (18%), Hungarian vizsla (12%), boxer (9%) and West Highland white terrier (8%). The most common positive test ((ELISA/IDT in %) reactions were given by the next allergens: *D. farinae*, *A. siro*, *D. pteronyssinus*, *T. putrescentiae*, ragweed and the trees in the first group (n_1=50), and *T. putrescentiae*, *D. farinae*, *A. siro*, *L. destructor* mugwort and ragweed in the second group (n_2=150) (**Table 1**). There was no test reaction in case of stinging nettle in the first group. 29 of 50 atopic dogs (58%) in the first group and 105 of 150 (70%) in the second group were positive in both the ELISA and IDT.

The positive correlations were the highest in the first group for *D. farinae*, *A. siro* and *D. pteronyssinus*; and in the second group for *T. putrescentiae*, *D. farinae* and *A. siro* (**Table 4/A-B**). The negative and total correlation was of course 100% where there was no positive test reaction (e.g. stinging nettle in the first group). *D. farinae*, *D. pteronyssinus*, *T. putrescentiae* and the trees showed the lowest total correlation at first time, and the *T. putrescentia*, *A. siro* and *D. farinae* secondly (**Table 4/A-B**). The average of total correlation was 92.3% and 85,83% in the first and in the second group, and the overall total correlation was 76% and 79% (**Table 4/A-B**).

The sensitivity and the agreement of the positive results were not valuable where there was no positive result either by ELISA or by IDT (for stinging nettle) (**Table 5/A, 6/A**). The sensitivity of ELISA by allergen was 100% for grassmix and *T. putrescentiae*, 93.2% and *A. siro.* in the first group, and 96% for mugwort, 91-90% for *L. destructor* and *T. putrescentiae* in the second group (**Table 5/A-B**). The sensitivity of ELISA was between 74.2-77.8% in ragweed, mugwort and *D. farinae*, the plantain and flea had the lowest sensitivity in the first measurements (**Table 5/A**). The lowest sensitivity had the *D. pteronyssinus* in the second group (**Table 5/B**). The sensitivity of IDT was 100% for *L. destructor*, plantain and mugwort, 95.8% for *D. farinae* in the first group, and 100, 89 and 88% for stinging nettle, *L. destructor* and ragweed (**Table 5/A-B**). The lowest sensitivity was for *T. putrescentiae* firstly, and *D. pteronyssinus* secondly (**Table 5/A-B**).

Table 4/A. Correlation in per cent of the ELISA test results with the IDT results by allergens in atopic dogs (n_1=50) (PC=positive correlation, NC=negative correlation, TC=total correlation).

Allergens (n_1=50)	+ELISA +IDT	-ELISA -IDT	+ELISA -IDT	-ELISA +IDT	PC (%)	NC (%)	TC (%)
					(+ELISA +IDT)/n	(-ELISA -IDT)/n	PC+NC
Dermatophagoides farinae	23	18	1	8	46	36	82
Dermatophagoides pteronyssinus	10	30	5	5	20	60	80
Acarus siro	14	30	5	1	28	60	88
Tyrophagus putrescentiae	3	40	7	0	6	80	86
Lepidoglyphus destructor	2	45	0	3	4	90	94
Grassmix	2	47	1	0	4	94	98
Ambrosia elatior (ragweed)	7	39	2	2	14	78	92
Urtica dioica (stinging nettle)	0	50	0	0	0	100	100
Plantago lanceolata Plantain	1	47	0	2	2	94	96
Artemisia vulgaris (mugwort)	5	43	0	2	10	86	96
Betula pendula/Alnus glutinosa/Corylus avellana (birch/alder/hazel)	5	38	3	4	10	76	86
Flea	1	46	1	2	2	92	94
Overall	29	9	1	11	58	18	76
Average					10.43	81.86	92.29

Table 4/B. Correlation in per cent of the ELISA test results with the IDT results by allergens in atopic dogs (n_2=150) (PC=positive correlation, NC=negative correlation, TC=total correlation).

Allergens (n_2=150)	+ELISA +IDT	-ELISA -IDT	+ELISA -IDT	-ELISA +IDT	PC (%)	NC (%)	TC (%)
					(+ELISA +IDT)/n	(-ELISA -IDT)/n	PC+NC
Dermatophagoides farinae	63	46	25	16	42	31	73
Dermatophagoides pteronyssinus	0	136	10	4	0	91	91
Acarus siro	56	46	30	18	37	31	68
Tyrophagus putrescentiae	65	34	44	7	43	23	66
Lepidoglyphus destructor	31	112	4	3	21	75	96
Grassmix	5	123	17	5	3	82	85
Ambrosia elatior (ragweed)	29	103	4	14	19	69	88
Urtica dioica (stinging nettle)	1	143	0	6	1	95	96
Plantago lanceolata Plantain	4	133	12	1	3	89	92
Artemisia vulgaris (mugwort)	27	113	9	1	18	75	93
Betula pendula/Alnus glutinosa/Corylus avellana (birch/alder/hazel)	4	131	14	1	12	87	99
Flea	12	112	9	7	8	75	83
Overall	105	13	27	5	88	9	97
Average					17,25	68,58	85,83

Table 5/A. Sensitivity of ELISA regarding IDT result as positive control by allergens and vica versa sensitivity of IDT regarding ELISA test result as positive control by allergens in atopic dogs; specificity of tests in the negative control dogs (k_1=19) by allergens.

Allergen k_1=19	Sn (ELISA) (%)	Sp (ELISA) (%)	Sn (IDT) (%)	Sp (IDT) (%)
	(+ELISA+IDT)/ ((+ELISA+IDT)+ (-ELISA+IDT))	-ELISA/k	(+ELISA+IDT)/ ((+ELISA+IDT)+ (+ELISA-IDT))	-IDT/k
Dermatophagoides farinae	74.2	94.47	95.8	94.47
Dermatophagoides pteronyssinus	66,7	100	66,7	100
Acarus siro	93,2	94.47	73,7	100
Tyrophagus putrescentiae	100	94.47	30	100
Lepidoglyphus destructor	40	100	100	100
Grassmix	100	100	66.7	100
Ambrosia elatior (ragweed)	77.8	100	77.8	100
Urtica dioica (stinging nettle)	-	100	-	100
Plantago lanceolata Plantain	33.2	100	100	100
Artemisia vulgaris (mugwort)	74.4	100	100	100
Betula pendula/Alnus glutinosa/Corylus avellana (birch/alder/hazel)	55.6	100	62.5	100
Flea	33.2	100	50	100
Average	68.9	98.82	74.8	99.61

Table 5/B. Sensitivity of ELISA regarding IDT result as positive control by allergens and vica versa sensitivity of IDT regarding ELISA test result as positive control by allergens in atopic dogs; specificity of tests in the negative control dogs (k_2=12) by allergens.

Allergen k_2=12	Sn (ELISA) (%)	Sp (ELISA) (%)	Sn (IDT) (%)	Sp (IDT) (%)
	(+ELISA+IDT)/ ((+ELISA+IDT)+ (-ELISA+IDT))	-ELISA/k	(+ELISA+IDT)/ ((+ELISA+IDT)+ (+ELISA-IDT))	-IDT/k
Dermatophagoides farinae	80	100	72	100
Dermatophagoides pteronyssinus	0	100	0	100
Acarus siro	76	100	65	100
Tyrophagus putrescentiae	90	100	60	100
Lepidoglyphus destructor	91	100	89	100
Grassmix	50	100	23	100
Ambrosia elatior (ragweed)	67	100	88	100
Urtica dioica (stinging nettle)	14	100	100	100
Plantago lanceolata Plantain	80	100	25	100
Artemisia vulgaris (mugwort)	96	100	75	100
Betula pendula/Alnus glutinosa/Corylus avellana (birch/alder/hazel)	80	100	22	100
Flea	63	100	57	100
Average	65,6	100	56,3	100

The agreement of positive test result was the highest for *D. farinae* and mugwort in the first group, and with mugwort and *L. destructor* in the second group (**Table 6/A-B**). Lowest was it for flea firstly and for *D. pteronyssinus* secondly. The agreement of positive results counted after Codner was the same as the sensitivity of ELISA by the allergens in our study (**Table 6/A-B, 5/A-B**). Agreement of negative test results counted both ways was the highest in stinging nettle in the first and second measurement (**Table 6/A-B**). Four dust mites (*D. farinae*, *D. pteronyssinus*, *A. siro*, *T. putrescentiae*) and trees had the lowest agreement in both evaluations of negative results in the first group, and three of those mites (*D. farinae*, *A. siro*, *T. putrescentiae)* in the second group (**Table 6/A-B**).

The two-tailed Pearson bivariate correlation was significant for each allergen as the p values were in each cases less then 0.01. Pearson's correlation coefficient and Kappa value showed strong positive association and/or excellent agreement in mugwort, grassmix, *D. farinae*, ragweed, *A. siro* in the first group and in *L. destructor*, mugwort, ragweed and flea in the second group (**Table 7/A,B**). The Pearson's correlation coefficient showed a stronger and significant correlation between ELISA to *D. farinae* and *D. pteronyssinus* (r=0.626) and significant but weak correlation between the IDT results to *D. farinae* and *D. pteronyssinus* (r=0.355). There was no correlation between them in the second group.

Adopting the assumption that the clinical diagnosis for atopic dermatitis was after Willemse's criteria 30 of the 50 atopic dogs (60%) had a positive ELISA test for at least one allergen, and 40 (80%) had a positive IDT for at least one allergen in the first group and 88% (ELISA), 73,3% (IDT) in the second group, respectively (**Table 2/A-B, 3/A-B**). Therefore overall sensitivity of the imovet biocheck panel and IDT was 60% and 80% firstly, and 88% and 73%, secondly. The specificities were in both test 94.47% firstly, and 100% secondly. The overall PPV, NPV were 96.77% and 47.37% in ELISA and 97.56% and 64.29% in IDT firstly, and 100% and 40% in ELISA and 100% and 23% in IDT secondly. Overall accuracy by ELISA was 69.57% and 84.06% with IDT in the first group, and 89% and 75% in the second group. Accuracy for flea in ELISA was 94% firstly and 83% secondly (**Table 2/A-B, 3/A-B**).

Discussion

Frequencies of allergens (**Table 1**)

The prevalence of skin test reaction is keepeng with those reported in other studies of atopic dogs in the UK and Europe (Sture et al. 1995; Mueller et al. 2000; Shaw and Day 2000; Reedy et al. 1997; Saevik et al. 2003), namely the high preponderance of reactions to house dust mite allergens and the smaller contribution from pollen allergens. The exception among pollens is the ragweed which is still being a relatively common allergen in Hungary (Tarpataki 2006). In USA both house dust and pollen allergens are important group of allergens (DeBoer 1989; Wassom and Grieve 1998). The proportion of test positivity is of course higher in those studies which consider the patient to have atopic dermatitis together with the presence of at least one strongly positive IDT (Lian and Halliwell 1998). There have been variations reported in the skin test response to dander derived from various species – in this study the response to danders was not investigated.

The major allergens positive in the ELISA test evaluated herein were the dust mites (*D. farinae*, *A. siro*, *D. pteronyssinus*, *T. putrescentiae*), ragweed, mugwort and pollen of trees. House dust, house dust mites (*D. farinae*, *D. pteronyssinus*) and human dander have in several studies been found to be major allergens in canine AD in Europe (Willemse and Van den Brom 1983; Vollset 1985; Sture et al. 1995; Saridomichelakis et al. 1999; Tarpataki et al. 2006). House dust mite is also the most important allergen in the United States and Asia according to ELISA tests (Masuda et al. 2000; Scott et al. 2001). In atopic dogs, positive skin test reaction to *D. farinae* are much more common than to *D. pteronyssinus* (Noli et al. 1996; Mueller 2000). Pollens appear to be less important allergens than house dust mites in canine atopy (Willemse and Van den Brom 1983; Vollset 1985; Sture et al. 1995). In the IDT and ELISA tests evaluated herein, there were several positive test results against pollens of ragweed, trees and mugwort.

Specificity and sensitivity (**Table 2/A-B, 3/A-B, 6/A-B**)

The commercial ELISA (imovet-biocheck) test analyzed in this study has not been assessed previously. The test showed an acceptable average (68.9% (m_1) and 65.6% (m_2)) sensitivity and a reduced (60% (m_1) then good (88% (m_2)) overall sensitivity but the average and overall specificities (98.82% and 94.47%) (m_1) firstly and 100% (m_2) secondly were excellent compared to other evaluations where overall specificity was 41.6% (Ginel et al.,1998); 91.6% (Mueller et al. 1999); 84.4% (Sævik 2003) and 0% (Codner 1993) (**Table 9**).

Table 6/A. Percentage of agreement between results of ELISA and IDT in the atopic dogs (n_1=50) counted in two different way demonstrated on the second row (Sp+ is the specificity of ELISA counted in the positive control group (not in the negative control group!)).

Allergen n_1=50	Agreement of positive test results (%)	Agreement of positive test results (counted as Codner (1993))= Sn ELISA (%)	Agreement of negative test results (%)	Agreement of negative test results (counted as Codner (1993)= Sp+ ELISA (%)
	(+ELISA+IDT)/ ((+ELISA+IDT)+ (+ELISA-IDT)+ (-ELISA+IDT))	(+ELISA+IDT)/ ((+ELISA+IDT)+ (-ELISA+IDT))	(-ELISA-IDT)/ ((-ELISA-IDT)+ (-ELISA+IDT)+ (+ELISA-IDT))	(-ELISA-IDT)/ ((-ELISA-IDT)+ (+ELISA-IDT))
Dermatophagoides farinae	72,7	74.2	80	94.74
Dermatophagoides pteronyssinus	50	66,7	83,1	85.71
Acarus siro	66,7	93,2	87,3	85.71
Tyrophagus putrescentiae	27,3	100	87,9	85.11
Lepidoglyphus destructor	40	40	95,5	100
Grassmix	66,7	100	98,5	97.92
Ambrosia elatior (ragweed)	63,6	77.8	93,5	95.12
Urtica dioica (stinging nettle)	-	-	100	100
Plantago lanceolata Plantain	33,3	33.2	97,1	100
Artemisia vulgaris (mugwort)	71,4	74.4	96,8	100
Betula pendula/Alnus glutinosa/Corylus avellana (birch/alder/hazel)	41,7	55.6	89,1	92.68
Flea	25	33.2	95,6	97.87
Average	50.76	68.9	93.17	95.96

Table 6/B. Percentage of agreement between results of ELISA and IDT in the atopic dogs (n_2=150) counted in two different way demonstrated on the second row (Sp+ is the specificity of ELISA counted in the positive control group (not in the negative control group!)).

Allergen n_2=150	Agreement of positive test results (%)	Agreement of positive test results (counted as Codner (1993))= Sn ELISA (%)	Agreement of negative test results (%)	Agreement of negative test results (counted as Codner (1993)= Sp+ ELISA (%)
	(+ELISA+IDT)/ ((+ELISA+IDT)+ (+ELISA-IDT)+ (-ELISA+IDT))	(+ELISA+IDT)/ ((+ELISA+IDT)+ (-ELISA+IDT))	(-ELISA-IDT)/ ((-ELISA-IDT)+ (-ELISA+IDT)+ (+ELISA-IDT))	(-ELISA-IDT)/ ((-ELISA-IDT)+ (+ELISA-IDT))
Dermatophagoides farinae	61	80	53	65
Dermatophagoides pteronyssinus	0	0	91	93
Acarus siro	54	76	49	61
Tyrophagus putrescentiae	56	90	40	77
Lepidoglyphus destructor	82	91	94	97
Grassmix	19	50	85	
Ambrosia elatior (ragweed)	62	67	85	96
Urtica dioica (stinging nettle)	14	14	96	100
Plantago lanceolata Plantain	24	80	91	92
Artemisia vulgaris (mugwort)	96	96	92	93
Betula pendula/Alnus glutinosa/Corylus avellana (birch/alder/hazel)	21	80	90	88
Flea	43	63	87,5	93
Average	44,3	65,6	79,5	79,6

Table 7/A. Correlation between results of different assays for reactions to all allergens (m_1=69). Result presented include Pearson's correlation coefficient (r) (level of statistical significance was set at 0.01), Kappa value and McNemar's matched chi-squared test (level of statistical significance was set at 0.05).

Allergens m_1=69 (ELISA/IDT)	Pearson's correlation coefficient (r)	Kappa value	McNemar's matched chi-squared test
Dermatophagoides farinae	0,75	0.734	0.039
Dermatophagoides pteronyssinus	0,574	0.574	1.000
Acarus siro	0,748	0.734	0.125
Tyrophagus putrescentiae	0,49	0.387	0.08
Lepidoglyphus destructor	0,618	0.553	0.250
Grassmix	0,81	0.793	1.000
Ambrosia elatior (ragweed)	0,744	0,744	1.000
Urtica dioica (stinging nettle)	-	-	-
Plantago lanceolata Plantain	0,569	0.489	0.5
Artemisia vulgaris (mugwort)	0,832	0.818	0.5
Betula pendula/Alnus glutinosa/Corylus avellana (birch/alder/hazel)	0,532	0.531	1.000
Flea	0,387	0.378	1.000
Average	0.641	0.612	0.59

Table 7/B. Correlation between results of different assays for reactions to all allergens (m_2=162). Result presented include Pearson's correlation coefficient (r) (level of statistical significance was set at 0.01), Kappa value and McNemar's matched chi-squared test (level of statistical significance was set at 0.05).

Allergens m_2=162 (ELISA/IDT)	Pearson's correlation coefficient (r)	Kappa value	McNemar's matched chi-squared test
Dermatophagoides farinae	0,498	0,495	0,211
Dermatophagoides pteronyssinus	-0,41 (no sign)	-0,37 (no sign)	0,000
Acarus siro	0,415	0,411	0,111
Tyrophagus putrescentiae	0,438	0,394	0,000
Lepidoglyphus destructor	0,871	0,871	1,000
Grassmix	0,273	0,249	0,017
Ambrosia elatior (ragweed)	0,703	0,692	0,031
Urtica dioica (stinging nettle)	0,371	0,242	0,031
Plantago lanceolata plantain	0,419	0,350	0,003
Artemisia vulgaris (mugwort)	0,816	0,806	0,021
Betula pendula/Alnus glutinosa/Corylus avellana (birch/alder/hazel)	0,391	0,315	0,001
Flea	0,545	0,544	0,804
Average	5,33	0,417	0,186

Interpretation of Kappa (Altman 1991):
Poor agreement= less than 0.2
Fair agreement= 0.20 to 0.40
Moderate agreement= 0.40 to 0.60
Good agreement= 0.60 to 0.80
Very good agreement= 0.80 to 1.00
Interpretation of Kappa (Streiner 1994):
Poor agreement<0.40
Fair agreement= 0.40 to 0.59
Good agreement= 0.60 to 0.74
Excellent agreement> 0.74

Interpretation of the correlation coefficient (r)
Strong negative association= -0.1 to – 0.7
Weak negative association= -0.7 to –0.3
Little or no association= -0.3 to +0.3
Weak positive association= +0.3 to +0.7
Strong positive association= +0.7 to +1.0

Compared with ELISA tests evaluated in earlier reports (Miller et al. 1992; Codner and Lesdsard 1993; Paradis and Lecuyer 1993; Bond et al. 1994; Bevier et al. 1997; Mueller 1999), the ELISA test employed in the present study seems to have a low overall (60% (m_1) and average (68.9% (m_1)) sensitivity, but higher then in the study (Saevik 2003) where the sensitivity of ELISA was 53.6% and Ginel's result (72.23% overall sensitivity) (1997) was not much more better either (**Table 9**). In the second step (m_2) the overall sensitivity was increased (88% (m_2)), reaching the result of Mueller and collegues (90.4%) (1999) (**Table 9**). A related ELISA test designed for use in practice has been evaluated in a recent study (Ginel et al. 1998). OD values higher than 0.150 were considered positive in that study, and the test showed a sensitivity of 72.2% and a specificity of 41.6%. The lower sensitivity and higher specificity found in the ELISA test evaluated herein could be due to the higher cut-off value defined by the laboratory. If a cut-off point is raised, specificity will increase, while sensitivity will decrease. To set an appropriate cut-off point is difficult and there is no standardised approach to determine the cut off point in ELISA allergy tests. It is also possible that it would be more correct to define an individual cut-off point for each allergen or allergen group. Moreover, during not accurate storage of sera the amount of allergen-specific IgE could be further reduced.

Sensitivity of *D. farinae* by ELISA in our study was lower (74.2% (m_1); 80% (m_2)) comparing to the other studies results (92.5% (Lian 1998), 95.1% (Mueller 1999) but the specificity of *D. farinae* by ELISA was higher (94.47% (m_1); 100% (m_2)) than in other measurements (44.4% (Lian 1998)) or similar to the results in Mueller's study (96.3%) (1999).

Sensitivity of *D. pteronyssinus* by ELISA in our study was lower (66.7%% (m_1)) and (0% (m_2)) comparing to other results (92.7% (Lian 1998) but the specificity was much more higher (100% (m_1;m_2)) than in other studies (29.4% (Lian 1998).

Sensitivity (74.4% (m_1); 96% (m_2)) and specificity (100% (m_1;m_2)) of mugwort were better by ELISA compared to 0%/96.3% (Lian 1998).

Sensitivity of flea by ELISA is very variable in the literature (94%, 78% (McCall et al. 1997), 50% (Lian 1998), in our study it was only 33.2% (m_1) and higher in second measurements (63% (m_2), but the specificity was excellent (100% (m_1;m_2)) opposite to the other results (49.4% (Lian 1998); 69%, 91% (McCall et al. 1997).

Circulating IgE immune complexes may interfere with IgE measurement by reducing diagnostic sensitivity (Vassella et al. 1990). This phenomenon is likely to influence monoclonal tests to a larger extent than polyclonal tests, since the one epitope recognised by the monoclonal antibodies could be masked by formation of immune complexes or this epitope may not be present any more (Peng et al. 1993). It is also possible that there is a population of atopic dogs with a disease that is not mediated by IgE, but certain subclasses of IgG. A reagenic antibody termed IgGd

has been identified in sera from atopic dogs and it has been suggested that this antibody plays a role in the pathogenesis of canine atopy (Willemse et al. 1985). This population of atopic dogs would not be detected in an ELISA test measuring allergen-specific IgE antibodies. However, a recent study questioned the role of IgGd in canine atopy (Lian and Halliwell 1998). In this study, the investigators found high levels of IgGd against *D. farinae* and *D. pteronyssinus* in healthy dogs, and the presence of IgGd directed against apparently irrelevant allergens in atopic dogs.

Finally, pruritic dogs and pruritic, non-atopic dogs with AFR may also have been incorrectly diagnosed as atopic, although great efforts were made to exclude other pruritic conditions among the atopic dogs.

Among the healthy and pruritic, non-atopic dogs with AFR, the frequency of positive ELISA test results was low (5.53% (m_1); 0% (m_2)) compared to other results (22.7%-58.4%) (Saevik et al. 2003; Ginel et al. 1998). There was no positive test reaction in dogs with AFR. Although, based on a relatively small number of dogs, this finding could indicate that the results of the ELISA and IDT evaluated in the present study are not markedly influenced by the occurence of a concurrent skin disease. Although higher levels were detected in atopic dogs than in normal animals without skin disease, the differences were not statistically significant in Halliwell's study (1998). Positive ELISA test results in a clinically normal dog could represent subclinical hypersensitivity or production of antibodies against ubiquitous antigens, and not a "true" false positive. Clinically normal dogs with high total IgE levels in the serum have been shown to have an increased number of positive ELISA test results (Griffin et al. 1990). The high level of IgE in normal dogs has been considered to be a result of the high incidence of parasite infestation in dogs (Paradis and Lecuyer 1993; Scott et al. 2001). All dogs were free from *Toxocara canis*, *Dirofilaria immitis* and *repens, Sarcoptes scabiei, Ctenocephalides spp.* as they had been treated by selamectin spot on solution twice, two months before sampling. But treatment with selamectin twice of course does not eliminate the circulating persistent antibodies.

To determine the potential value of the IDT as a diagnostic method numerous factors that may influence the test results have to be considered. There have been positive reactions to antigens when by skin testing of clinically normal dogs (August 1982a; Willemse and Van Den Brom 1982; Codner and Tinker 1995; Lian and Halliwell 1998), especially with house dust and house dust mite allergens. Our results show much better specificity because there was only one positive IDT reaction only for *D. farinae* in negative control group (5.53% (m_1)) which contained non-atopic dogs with AFR too, and 0% (m_2)) in second measurement opposite to the results of Codner and Trinker (1995), and Lian and Halliwell (1998), where the 50-58% and 91.7% of clinically normal dogs were sensitive to *D.farinae* during IDT. It is important to use an appropiate concentration of

the allergen to distinguish between normal and atopic dogs, and to avoid false positives and false negatives.

All of the positive control dogs (50+150) in the study reported herein were considered to fulfill the criteria for a diagnosis of atopic dermatitis and yet 20% (10/50) (m_1) and 27% (40/150) (m_2) were IDT negative. It is difficult to comment about the likelihood that they had false negative IDT results – there is no means of determining the accuracy of the IDT only the provocative challenge. DeBoer (1989) reported up to 20%, and Foster (2003) up to 16% negative/equivocal IDT results in suspected atopic dermatitis. Hillier and DeBoer (2001) review the factors that may lead to false negative results, this may include, amongst others, drug interference and inherent host factors. In six cases stress and pseudogravidity were considered to have potentially influenced the results of IDT, steroid therapy was excluded. The test is still considered useful and for many veterinarians is the preffered diagnostic technique (August 1982b; Willemse and Van Den Brom 1983; Reedy et al. 1997; Scott et al. 2001). Skin testing and serological assays measure different components of an IgE response to an antigen. The skin test reaction demonstrates IgE bound to the mast cell via FcεRI. The half-life of these antibodies varies, for example, in humans, mast cell bound IgE has a half-life of up to 14 days compared with 2.3 days for serum IgE (Ishizaka and Ishizaka 1975). IDT may remain positive for months after serum levels of allergen-reactive IgE have waned, because when systemic levels of IgE become low, the remaining antibodies are probably bound on the surface of mast cells in the tissues rather than circulating in the blood in dogs, too (Wassom and Grieve 1998). Indeed, there may be various kinds of canine IgE that have different biological properties (Peng et al. 1997; Halliwell et al. 1998; Lian and Halliwell 1998; Scott et al. 2001; Hillier and DeBoer 2001). Consequently it would not be surprising if the results of a serological assay did not correlate highly with those of an IDT. Another major factor to consider is that the antigens used were from different sources, consequently it is unknown how much different the test results would have been, had the same antigens been used in both tests.

Perhaps, it is not expected that all dogs with a clinical diagnosis of AD will show positive results on an allergy test – other factors seemingly unrelated to IgE-mediated hypersensitivity may explain the clinical signs. Namely, physician allergists now recognize two different forms of AD in humans (DeBoer 2003). In the “extrinsic” form, there is a family history of allergy and positive skin and serum allergen-specific IgE tests. In the “intrinsic” form, patients have identical clinical symptoms and a family history of allergy yet do not show positive results with the same “allergy tests” (Novak 2002)

The overall positive predictive values of the ELISA and IDT were high (96.77% and 97.56% (m_1) and 100-100% (m_2)) and the overall negative predective values were low by IDT (64.29% (m_1)) and very low 23% (m_2)); by ELISA low (47.37% (m_1); 40% (m_2)), respectively

(**Table 2/A-B, 3/A-B**). Thus these results are mostly higher then compared to other evaluations where overall PPV and NPV of ELISA were 76.47% and 35.71% (Ginel et al., 1998), and the average PPV of ELISA was 63.2% (Lian 1998), but the average NPV in last study was higher (80%) than our overall NPVs. Our findings may indicate that this ELISA and IDT have the value in differentiating between atopic and non-atopic dogs and should be used to confirm a diagnosis of canine atopy. But it is not allowed to calculate the positive and negative predective values on samples where the prevalence of the disease was artificially and not naturally controlled (herein 50 /19 (m_1) and 150/12 (m_2) atopic/non-atopic dogs). In a study, evaluating PPV and NPV for atopic dermatitis should artificially recruited healthy and diseased patients in a 90 to 10 ratio, then the natural prevalence of the disease is 10% in the canine population (Scott et al. 2001).

Agreement (**Table 6/A-B**)

28 of 50 (56%) (m_1) and 105/150 (70%) (m_2) atopic dogs were positive in both the ELISA and IDT which is similar (m_1) and higher (m_2) than Sævik's result (53.6%) (2003). Of these 28 dogs, 12 (42.9%) (m_1) gave similar positive test results for all examined allergens in both tests. There were less, only three atopic dogs (20%) where test results were the similar in both tests in the mentioned study (Sævik 2003).

The percentage of agreement between results of ELISA and IDT in the atopic dogs was compared to Codner's similar results (**Table 8**). All our results were higher then Codner's ones with the exception of flea where the agreement of positive test results (33.2%) was higher in Codner's (45%) (1993), but it was improved in our second measurements and became higher (63%) than Codner's. It is worth to mention that the „agreement between IDT and ELISA for positive and negative results" after Codner was called in other and our studies as total correlation (TC) (Ginel 1998). Range of total correlation was the strongest in our studies (73-100%), lower was in Ginel's study (47.1%-80.4%) and the weakest was in Codner's measurements (22%-56%) (**Table 9**). The „agreement of positive test results" after Codner (1993) is identical with the sensitivity of ELISA counted by allergen in Lian and Halliwell's (1998) and our studies. The „agreement of negative test results" after Codner is the same as the specificity of ELISA counted by allergens. The average Kappa values (0.612 (m_1) and 0.417 (m_2)) were higher too than Codner's (0.17).

The top ranges of positive and negative correlation (PC (1%-46%) and NC (23%-95%) were higher than described by Ginel (PC (2.7%-19.4%) and NC (41.6%-63.8%) (1998) (**Table 9**).

Table 8. Percentage of agreement between results of ELISA and IDT in the atopic dogs (in the first row: n_1=50; in the second row: n_2=150) compared to Codner's similar results.

Allergen In the first row: n_1=50; in the second row: n_2=150	Agreement of positive test results (%); results of Codner (1993)	Agreement of positive test results (counted as Codner (1993))= Sn ELISA (%) Our results	Agreement of negative test results (%); results of Codner (1993)	Agreement of negative test results (counted as Codner (1993)= Sp+ ELISA(%) Our results	Agreement of positive and negative test results; results of Codner (1993)	Agreement of positive and negative test results; counted as Codner (1993)= TC (%) Our results
	(+ELISA+IDT)/ ((+ELISA+IDT)+ (+ELISA-IDT)+ (-ELISA+IDT))	(+ELISA+IDT)/ ((+ELISA+IDT)+ (-ELISA+IDT))	(-ELISA-IDT)/ ((-ELISA-IDT)+ (-ELISA+IDT)+ (+ELISA-IDT))	(-ELISA-IDT)/ ((-ELISA-IDT)+ (+ELISA-IDT))	Percentage of dogs for which IDT and ELISA results agreed	PC+NC
D. farinae, D. pteronyssinus (m1: in the first row; m2: in the row belove)	73	74.2; 66,7 80; 0	66.7-74.2	94.7; 85,7 65-93	39	82; 80 73;91
Pollens (Grassmix, ragweed, stinging nettle, Plantain, mugwort, birch/alder/hazel) (m1: in the first row; m2: in the row belove)	75-100	33.2-100 14-96	34-53	92.7-100 88-100	44-56	86-100 85-99
Flea (m1: in the first row; m2: inthe row belove)	45	33.2 63	78	98 93	54	94 83

Table 9. **Results of the ELISA test in per cents (%) (Sp=specificity, Sn=sensitivity, PPV=positive predective value, NPV=negative predective value, Acc=accuracy, PC=positive correlation, NC=negative correlation, TC=total correlation).**

ELISA	Sp overall/ average	Sn overall/ average	PPV overall/ average	NPV overall/ average	Acc overall/ average	range of PC by allergens	range of NC by allergens	range of TC by allergens
this study (m_1=69)	94.47/ 98.82	60/ 68.9	96.77/ -	47.37/ -	69.57	2-46	36-100	80-100
this study (m_2=162)	100/ 100	88/ 65,6	100	40	89	0-43	23-95	66-99
Bevier 1997	-/ 92	-/ 86			-/80-98 (range) 90			
Lian 1998	-/ 72.25	-/ 45.72	-/ 63.2	-/ 80				
Codner 1993	0/ -	100/ -						22-56
Ginel 1998	41.6/ -	72.23/ -	96.77/ -	43.37/ -		2.7-19.4	41.6-63.8	47.1-80.4
Mueller 1999	91.6/ -	90.4/ -						
Sævik 2003	84.4/ -	53.6/ -	75/ -	67.5/ -				
Foster 2003	64.2-96.6 (range)	19.3-77.1 (range)						

Conclusion

The evaluated improved ELISA test seemed reliable for the diagnosis of atopy in practice comparing to the other commercially available tests and can be recommended for use in dogs when immunotherapy is a therapeutic option. The aim of the development was to increase the sensitivity of the imovet bg allergy-serology test. The overall sensitivity and sensitivities by allergen in 7 of 12 allergens (58%) were increased (**Table 2/A-B; 5/A-B**). The positive correlation and agreement of positive test results increased in 67% and 50% of examined allergens (**Table 4/A-B; 6/A-B**).

There is no doubt, that it is very difficult to evaluate an ELISA and an IDT in a correct way. There are lots of calculating-methods which evaluate the different allergy-test methods. The aim of the conclusion is to consider the best evaluation which would be the most correct way in the future.

The overall values of specificity, sensitivity positive and negative predective values, accuracy are only good to get a general impression of the tests (both ELISA and IDT) then the results by allergens in the same dog may be totally different (if the same dog's sera is positive only for *D. farinae* but the IDT showed positive reaction to *L. destructor* only, the overall evaluation

does not reflect the differencies, only that this dog was positive by both tests). In such way we can get a good overall specificity and sensitivity of a test although the correlations examined by allergens are zero.

Comparing tests by allergens is a more accurate way of valuation. The problem with this evaluation is that the positive control dogs for atopic dermatitis are sensitive not to all examined allergens, and even if they are atopic they normally will give negative reactions to several allergens. These reactions are then misinterpreted as “fals negative” reactions. The real positive control dog sensitive for an allergen would be the dog which would improve after a total environmental change and would relapse after specific (simple allergen) provocation. This method is not usefull but would give an accurate causal diagnosis and positive control for one allergen. The only choice is to compare tests with each other. IDT has become “the golden standard” at evaluations of allergy serology tests. Well, inspite that IDT is a provocation test, and shows the presence of allergen-specific IgE antibodies on the mastocytes, many healthy dogs may give positive intradermal skin test reactions to one or more allergens. That may reduce the “absolute positive control role” of IDT by allergens. Without other available correct provocation test IDT is the “golden standard” of evaluating an allergy serology test.

Evaluation of tests by allergens is more correct when we take into consideration all the positive reactions by allergens given by both test methods. The more single positive reactions examined by one allergen by both tests in the positive control dogs there are, the less accurate is the test. The number which describes this the best is the “accuracy” counted by allergens (or the “total correlation=TN”, which means the same).

Regarding allergy serology it exams an earlier state of an allergic immune response (the production of allergen-specific IgE antibodies in the phase of sensitivisation) then IDT does (allergen-specific IgEs are bound on mastocytes’ Fc receptors and mastocytes are degranulated if the appropiate allergens arrive). That is why serology will be always more sensitive as IDT.

The other point of positive control: the concept of false positive and false negative reactions in allergy testing is conflicting. If the test is used to confirm a diagnosis, as many manufacturers promote it, the absence of positive reactions in an atopic dog may be considered as false negative. A positive reaction may only be considered strictly as false positive if the dog has never contacted the specific allergen (Reedy and Miller 1989) because a positive result in a normal dog may reflect subclinical sensitization. However, if the test is used to confirm a diagnosis, such a subclinical reaction would lead to an incorrect diagnosis. Thus it should be considered a false positive, and the test modified to discriminate between clinically relevant and irrelevant reactions. Since the IDT may produce both false negative and false positive results, it cannot be used as the reference method

to evaluate the *in vitro* tests (Bond et al. 1994) where diagnostic value has to be based on the percentage of agreement with clinical diagnosis.

The positive predective value of a test is the probability that the patient has the disease when restricted to those patients who are tested positive. It is not suggested to calculate the positive predictive value on a sample where the prevalence of the disease was artifically controlled, as it was in our study.

Which data are important, informative and exact in evaluation that is the main question in the future. The sensitivity and specificity of an allergy-serology (regarding the intradermal skin test results as the "golden standard" for positive control) counted by allergen still remain an accepted and always evaluated aspect of an ELISA test. The positive, negative and total correlation counted by allergen is relatively good description of test-correlation although they depend on the allergen-proportion (since for example if there is no positive reaction the NC and TC are 100%, which may not reflect the real value of the test). The overall values of specificity, sensitivity and accuracy are only sufficient to get a general impression of the tests (both ELISA and IDT) then the results by allergens in the same dog may be totally different. The author suggests to evaluate the agreement of positive { (+ELISA+IDT) / ((+ELISA+IDT) + (+ELISA-IDT) + (-ELISA+IDT)) } test results of each allergen (**Table 6/A-B**) in the future in each allergy-serology. The reason is that these values would reflect the most exact correlation of any positive reaction since all evaluations are depending on the prevalence of a positive reaction to allergens (the more positive reactions there are in both tests the higher the possibility of variances in test results may occur). In each allergen the prevalence of it in percentage should be given (then if there was no positive reaction we should know that the 0 agreement is not the failure of the test).

In conclusion, both types of tests measure a different aspect of the atopic disease, and a 100% correlation cannot be expected.

Figure 1. Lab Equipment for IMOVET allergy-serology.
(**www.laupeneck.ch**)

Figure 2. Tubes for blood sample (plastic tubes and BD Vacutainer™ SST).

N. Tarpataki, B. Bigler, P. Vajdovich, K. Vörös: Evaluation of an enzyme-linked immunosorbent assay (ELISA) for serological diagnosis of canine scabies. Under (second) reviewing process in *Journal of Small Animal Practice.*

Introduction

Infection with *Sarcoptes scabiei* var. *canis* occurs commonly in dogs. It often causes a severe skin disease which is difficult to diagnose, particularly in the acute stage of infection. The *Sarcoptes*-infestation has remained a consistent problem over the years occuring with variable frequency. It is the major consideration in the differential diagnosis of pruritic dermatosis in dogs. *Sarcoptes*-dermatosis is highly contagious infection but marked individual variation in disease expression with the possibility of asymptomatic carriers. Infection generally results from direct contact but sometimes by indirect contact with the origin of the disease remaining obscure. There is a possible contagion to humans, where zoonotic lesion (pruritic papules on the trunk, arms and legs) are common. However, as the parasite is not well adapted to human skin, adult rapidly die and cannot reproduce; affected individuals normally recover spontaneously once the animal is successfully treated. Most commonly found in puppies and young dogs, however, it also occurs in adult dogs. The typical clinical picture of scabies, as described in literature, includes intense pruritus, alopecia and crusting papules on predilection sites, such as ear margins (**Figure A**), elbows (**Figure B**) and parts of the distal legs (Scott et al. 2001, Bourdeau et al. 2004). However, numerous patients with generalised pruritus show little or no evidence of the aforementioned skin lesions. This makes it difficult to diagnose scabies definitely without further examination. A reliable diagnostic method should provide the possibility to differentiate scabies from all other forms of allergic dermatitis especially from atopic dermatitis or food allergy, that may be quite similar regarding the clinical signs. Using skin scrapings from affected body areas is not a sensitive method, as mites are found in only 22.8-50% of the samples (Bourdeau et al. 2004). Histopathology of the affected areas is not specific either if mites are not found. The indirect way of diagnosing the disease is treatment with acaricidal agents (Scott et al. 2001).

The aim of this study was to evaluate the Swiss scabies ELISA test (IMOVET sarcoptes) in the diagnosis of canine scabies and differential diagnosis of atopic dermatitis. To our knowledge, there is no study comparing the results of *Sarcoptes*-serology and allergy-serology (measurement of allergen-specific IgE for other mites) in clinical patients with *Sarcoptes* infestation. The cross-reaction between allergens of *D. farinae*, *D. pteronyssinus* and immunogenic proteins of scabies mites has been demonstrated (Prelaud and Guagere 1995, Arlian et al. 1991). However, cross-reaction between scabies mites and *A. siro*, as well as *T. putrescentiae* has not yet been verified. The sarcoptes-specific IgE was measured to examine the hypersensitivity reaction to the *Sarcoptes* mite.

Materials and methods

After dermatological examination, blood sera and skin scrapings were taken from 36 dogs. Twenty-nine of the 36 were pruritic, with a tentative diagnosis of *Sarcoptes scabiei* infestation. All the patients (29) were referred by vets to the dermatology unit of our Department between January and August 2003. Clinical symptoms were described and the intensity of pruritus was evaluated and scaled into 5 grades. Grade 5/5 denotes the most intense pruritus (continuous self-trauma).Grade 1/5 denotes a dog's normal self-cleaning. Skin scrapings were taken from each patient and examined under a light microscope. If pyoderma or fungi/yeast were present clinically and in the bacterial and

fungal culture, the patient was treated with the appropriate antibiotic or antimicotic drug for at least 3 weeks. Anti-inflammatory drug therapy was discontinued, including oral and topical glucocorticoids and anti-histamines for at least 4 weeks and 10 days, respectively, before further sampling. Blood samples were taken from all dogs using BD Vacutainer™ SST (serum sample tube). The sera were separated, frozen immediately and stored at -17 °C until they were sent to Labor Laupeneck. They were then tested for *Sarcoptes*-specific IgG-antibodies (*Sarcoptes*-IgG) and *Sarcoptes*-specific IgE-antibodies (*Sarcoptes*-IgE), as well as IgE-antibodies specific to *D. farinae*, *D. pteronyssinus*, *A. siro* and *T. putrescentiae* (allergy-serology). The sera were analyzed using the indirect ELISA test (IMOVET sarcoptes) containing a polyclonal sheep antidog-IgG, conjugated with alkaline-phosphatase for measuring *Sarcoptes*-IgG, while a monoclonal antibody D9 was used to measure the *Sarcoptes*-IgE. An intradermal skin test (IDT) was performed on just 16 dogs, using the Artuvetrin Test solutions, and histopathological examinations were conducted on just 6 patients. We wanted to carry out the IDT and skin biopsy on all 36 dogs, but were unable to do so because the owners did not co-operate. On 8 patients, the lateral thorax skin as the site for IDT was not free from lesions.

Scabicidal therapy, using a 0.025% (250 ppm) amitraz solution (Taktic©; Hoechst Roussel Vet GmbH, Rheingaustrasse 190, D-65203 Wiesbaden, Germany) for local treatment (once or twice a week for 4-6 weeks), or selamectin (Stronghold® spot on) for systemic treatment (twice with a 4 week interval), was prescribed and evaluated after two months' therapy for all (29) patients. Ectoparasitic skin diseases other than *Sarcoptic acariosis* (otodectic dermatitis, cheyletiellosis, Pelodera dermatitis, louse infestation, etc.), which could also have responded to parasiticidal therapy, were ruled out by clinical and microscopic examinations of the skin scrapings (Scott et al. 2001).

If one of the typical clinical signs was present and/or the dog had demonstrable mites in the skin scraping, and it responded to 2 months of acaricid treatment, this served as the ‘gold standard’ for the diagnosis of infection with *Sarcoptes* mites (Bornstein et al. 1996). We considered (n=)17 dogs as a positive control group that improved markedly (skin lesions disappeared, pruritus grade 1/5) after 2 months of scabicidal therapy.

Twelve dogs did not have demonstrable mites on the skin scraping and the result of scabicidal treatment was partially effective after 2 months of scabicidal therapy. Scabicidal therapy was partially effective when the skin lesions had not disappeared completely and/or pruritus was reduced, but not to grade 1/5. After 2 months of scabicidal therapy, an 8 week elimination monodiet was prescribed to demonstrate any adverse reaction to food (ARF). Three of the (12) allergic dogs responded to the elimination monodiet (Table 1). Willemse's diagnostic criteria are traditionally arranged into major and minor criteria. If 3 major and 3 minor criteria are determined, and

diagnoses other than atopy have been ruled out, an atopic dermatitis diagnosis can be made (Willemse 1986). According to Willemse's criteria, we suspected an atopic dermatitis diagnosis in 9 patients which did not respond to the elimination monodiet. These 9 dogs had atopic dermatitis, with or without *Sarcoptes* infestation, at the first examination.

The negative control group consisted of 7 non-pruritic blood-donor dogs without clinical signs of dermatitis for at least 2 months, and with negative skin scrapings for scabies.

The specificity and sensitivity of the diagnostic methods were calculated. The specificity of the test methods was taken just once in the negative control group (7 dogs), and together with the allergic patients, as in Bornstein et al. (1996), also included all the dogs not responding to acaricidal therapy.

Results

The results of the examinations are shown in **Table 1**.

Negative control group:

One dog in the group reacted positively to both *Sarcoptes*-IgG and allergy-serology, thus the specificity of ELISA for *Sarcoptes*-IgG (in the negative control group) was 85.7%. Two dogs had positive results with *Sarcoptes*-IgE, giving a specificity of 71.4% (in the negative control group). Five dogs reacted positively to allergy-serology for one or more mites without any clinical symptoms.

Positive control group:

Microscopic examination showed *Sarcoptes scabiei* in just two dogs' samples. Thus, the sensitivity of microscopic examination for scabies was 11.8% (specificity: 100%).

Histopathological examination showed inflammatory changes of the skin typical for ectoparasitic infestation. Sixteen out of 17 dogs reacted positively to the *Sarcoptes*-IgG. The sensitivity of ELISA for *Sarcoptes*-IgG was 94.1%. Four of the 16 patients with positive *Sarcoptes*-IgG reacted positively to allergy-serology for one or more allergens. In 4 cases, IDT showed a positive reaction for one or more allergens. Only one patient in the positive control group had a positive *Sarcoptes*-IgE result (sensitivity: 5.8 %).

Allergic patients:

These patients were pruritic, the skin scraping examination was negative, and the scabicidal treatment was partially effective. At the first examination, three of them had AFR and nine atopic dermatitis, with or without *Sarcoptes*-infestation. Five of them showed positive results to *Sarcoptes*-IgG, and 7 were negative. In 6 cases, both IDT and allergy-serology were positive for one or more mite allergens. None of them reacted positively when measuring the *Sarcoptes*-IgE. If we calculate the specificity, as Bornstein et al. (1996) did, including all the dogs not responding to

acaricidal therapy as being non-infected, the specificity of *Sarcoptes*-IgG drops to 68.4% and that of *Sarcoptes*-IgE rises to 89.4%.

Discussion

Sarcoptes-IgG

Scabies infestation causes the production of measurable IgG- and IgM-antibodies in infested species, humans (Arlian et al. 1994), dogs (Bornstein and Zakrisson 1994, 1996, Arlian et al. 1996), pigs (Bornstein and Zakrisson 1993, Hollanders et al. 1997, Van der Heiden et al. 2000) and cattle (Fischer et al. 1986). Our study showed that the IMOVET sarcoptes test is sensitive (94.1%) enough for the detection of antibodies to *Sarcoptes scabiei* in dogs. The specificity (68.4%) (calculated as Bornstein et al. did) is not accurate enough to differentiate allergic skin diseases. The specificity (85.7%), calculated with just the negative control dogs, and sensitivity are comparable to results obtained in the United States using the same IMOVET sarcoptes test, with sensitivity and specificity of 84.2% and 89.5%, respectively (Lower et al. 2001), and to another recent similar study in the United Kingdom using the Swedish scabies ELISA test (sensitivity: 83%, specificity: 92%) (Curtis 2000), although in these studies, microscopic identification of scabies mites (and/or ova) was used as the 'gold standard' of diagnosis, in contrast to our study and another Swedish one (sensitivity: 92%, specificity: 96%) (Bornstein et al. 1996). Diagnosis of canine scabies by response to scabicidal treatment is clinically more realistic, since mites are found in only a few infested dogs' skin scrapings (Bornstein et al. 1996, Bourdeau et al. 2004). Because of the low sensitivity (in our study: 11.8%), skin scraping used alone is an inadequate method for diagnosing scabies. Histopathological examination could demonstrate inflammatory changes in those cases typical for ectoparasitic infestation, but without the presence of any *Sarcoptes* mites they were not pathognomic (Scott et al. 2001).

The sensitivity of the IMOVET sarcoptes test in our study was 94.1% because one dog had no measurable antibodies, despite demonstrable mites on its skin scraping and response to scabicidal treatment. This dog had had symptoms for 5 months and been treated with glucocorticoids, which had been stopped four weeks before sampling. This dog had measurable *D. farinae* and *T. putrescentiae*-specific IgE-antibodies. In general, seroconversion occurs rapidly in *Sarcoptes* infection. Dogs infested with a relatively high or low dose of *S. scabiei* seroconverted 2 and 4-5 weeks post infection (Bornstein and Zakrisson 1994). When dogs were experimentally infected with a relatively low dose of *S. scabiei,* seroconversion occurred 4-5 weeks post infection in half the dogs. Clinical signs and antibodies, not to *S. scabiei,* developed in the other half just 2 weeks after a re-infection (Bornstein and Zakrisson 1994). The dogs mentioned may have had some other pruritic disease at the outset and could have become infested by a low dose of sarcoptic mange in the meantime. One potential reason for this false negative reaction to *Sarcoptes*-IgG may be the lack of sufficient time for seroconversion to occur, if the infective dose of *Sarcoptes scabiei* was really low and infection took place less than 4-5 weeks before sampling in these dogs (Bornstein and Zakrisson 1994). Another potential cause for the false negative titre may have been a laboratory error. In this case, a repeated sampling would have been needed.

The specificity of the IMOVET sarcoptes test in our study was 85.7% due to one positive result in the negative control group, and 68.4% if calculated with all the dogs in the positive control group (Bornstein et al. 1996). The dogs in the negative control group were well controlled, non-pruritic, without any skin lesions, and had had a negative skin scraping test for at least 2 months. One possible cause of the false positive reaction may be that measurable antibodies to scabies mites were found 1-4.5 months after successful treatment of natural infection in studies of human patients (Arlian et al. 1994) and dogs (Bornstein and Zakrisson 1994, Arlian et al. 1996). These dogs might have had scabies infestation and treatment earlier. The other possible cause of the false positive serologic results for *Sarcoptes scabiei* may be cross-reactivity with antibodies to house dust mites (*D. farinae* and *D. pteronyssinus*), which have been shown *in vitro* and *in vivo* to share antigens with *Sarcoptes scabiei* in humans (Falk et al. 1981, Arlian et al. 1991, 1995) and dogs (Arlian and Morgan 2000).

Using crossed immuno-electrophoresis and SDS-PAGE (sodium-dodecyl-sulfate-polyacrylamide gel electrophoresis), it was shown that 6 out of 9 *Sarcoptes scabiei*-antigens cross-reacted with IgE-antibodies when incubated in the sera of patients sensitive to *D. farinae* without scabies infestation (Arlian and Morgan 2000). This is possible in this false positive case, because IgE antibodies to *D. farinae*, *A. siro* and *T. putrescentiae* were detected in its sera, too. Alternatively, some dogs may have elevated serum *Sarcoptes*-IgG and *D. farinae*-specific IgE antibody levels, even though they are clinically healthy.

The results of the IMOVET sarcoptes test in the 'allergic patients' group were positive in 5 cases and negative in 7. This is the group of pruritic dogs without demonstrable mites in their skin scrapings, and scabicidal treatment was partially effective. Six of them reacted positively to allergy-serology for some allergens, and 7 of them had some positive IDT reaction, too. Nine of the 12, the atopic dogs, met Willemse's criteria and three of them, the dogs with AFR, responded to the elimination monodiet.

Five atopic patients with positive reactions (two of them with positive allergy-serology (*D. farinae*, *A. siro* and *T. putrescentiae*)) might have had both atopic dermatitis and *Sarcoptes* infestation and, if so, then the *Sarcoptes*-IgG was relevant. The lack of marked response to scabicidal treatment might be because of concomitant atopic dermatitis and the fact that individuals sensitive to dust mites may be more prone to scabies and show more severe clinical signs than non-allergic individuals. This occurs in human patients (Arlian et al.

1991) and dogs (Curtis 2000), too. Thus, scabicidal treatment alone did not suffice in these cases. If they had only had atopic dermatitis without *Sarcoptes* mites, the cause of the false positive Sarcoptes-serology might have been cross-reactivity with antibodies to dust mites (*D. farinae*, *A. siro* and *T. putrescentiae*). The first two have been shown to share common antigens with *S. scabiei* in humans (Moustafa et al. 1998), but *A.siro* has not been examined in dogs either *in vitro* or *in vivo* yet, but only in pigs (Van der Hejiden et al. 2000).

Conversely, if they had had only *Sarcoptes* infestation, the allergy tests would have given false positive results. They might not be really falsely positive, because the dogs developed new IgE-antibodies to *D. farinae* and *D. pteronyssinus* after infestation with scabies, even though they were not exposed to these mites. Response to such allergens may explain the persistence of symptoms in some cases after treatment for scabies in dogs (Arlian and Morgan 2000). There is no similar study examining the development of sensitivity to *A. siro* or to *T. putrescentiae* in canine scabies.

Seven patients with a negative *Sarcoptes*-IgG result in the allergic group did respond, but not satisfactorily, to scabicidal treatment. Four of them had measurable allergen-specific IgE to some allergens and also positive IDT reactions. They might have responded to acaricidal treatment because the allergen-level, the amount of dust and storage mites on the dogs, was reduced by acaricidal therapy.

Allergy-serology and Sarcoptes-IgG

Single results were compared as we were interested in the immunological reactions. The Sarcoptes-IgG test showed a positive reaction in 22 cases; 27.3% of them were positive for *D. farinae* –specific-IgE, 22.7% to *A. siro*, 27.3% for *T. putrescentiae*, and much lower, 4.5% for *D.pteronyssinus*, too. In a study (Prelaud and Guagere 1995), 60% of dogs infested with *S. scabiei* showed a positive reaction to *D. farinae*, but this reaction was not seen again after complete recovery following treatment for *S. scabiei*, and none of these dogs showed any signs of atopy in the following three years. It is highly possible that dogs infested with *S. scabiei* are also sensitized to *D. farinae* and *T. putrescentiae,* even though they have no history of atopy, as is shown in a study of humans (Moustafa et al. 1998). In the same study, 25 non-atopic patients were examined for their reaction to exposure to house dust and *T. putrescentiae* antigens using the skin prick test. According to their results, human patients with a history of concurrent scabies infestation showed significantly higher positivity than non-scabietic patients (Falk and Bolle 1980a, Arlian et al. 1991). These might

confirm the existence of *in vivo* cross-reactivity between *S. scabiei*, *D. farinae*. and *T. putrescentiae*.

Although *A.siro* in dogs has not been shown to share antigens with *S. scabiei,* the cross-reactivity is also high. The possible cause for the lower 'co-positivity' with *D. pteronyssinus* may simply be the lower incidence of *D. pteronyssinus*–allergy in Hungary; there were only three positive cases in our study.

Conversely, among all the 36 dogs examined, a positive Sarcoptes-IgG test was shown in 46% of those sensitive to house dust mite and, similarly high, 55.5% of those sensitive to *A. siro*, and 42,8% of *T. putrescentiae* sensitive dogs. These results were higher than those of others investigating the Swedish scabies ELISA who found no positive scabies results in 12 house dust mite-reactive atopic dogs (Curtis 2000).

Positive Sarcoptes-IgE reactions

The measurement of *Sarcoptes*-IgE is not an acceptable method for diagnosing either *Sarcoptes* mange infestation or *Sarcoptes*-allergy in dogs. There were only 3 positive cases which may have been due to cross-reactions with the dust mites antigen. Common antigens were shown in a study of sera from 83 dogs, evaluating them using SDA-PAGE and immunoblotting to identify proteins in extracts prepared from the dust mites *D. farinae*, *D. pteronyssinus*, *Euroglyphus maynei* and *S. scabiei var. canis*, binding to Ig-E antibodies. It was found that some dogs with serum-IgE against dust mites also had IgEs against antigens of *S. scabiei* var. *canis* (Schumann et al. 2001).

Table 1: Results

Columns: number of dogs and the results of each examination

Lines: all the results of each dog

List of abbreviations: D.f. (Dermatophagoides farinae), D.p. (Dermatophagoides pteronyssinus), A.s. (Acarus siro), T.p. (Tyrophagus putrescentiae), th (respond to scabicidal therapy), ss (skin scraping), sb (skin biopsy), IDT (intradermal skin test), IgE/IgG (measurement of these antibodies), + (positive), - (negative), lack of a cell (there was no examination). Positive serology results are indicated with the numbers 3,4,5, (which are in correlation with the OD), the negative results with 1,2.; AD (atopic dermatitis); AFR (adverse food reaction)

Dogs	**D.f.**	**D.f.**	**D.p.**	**D.p.**	**A.s.**	**A.s.**	**T.p.**	**T.p.**	**Sarcoptes**	**Sarcoptes**	**th**	**ss**	**Sb**
number	**IDT**	**IgE**	**IDT**	**IgE**	**IDT**	**IgE**	**IDT**	**IgE**	**Ig-G**	**Ig-E**			
Positive control patients													
1	**+**	**4**	**-**	**1**	**+**	**3**	**-**	**3**	**positive**	**3**	**+**	**-**	
2	**+**	**3**	**-**	**5**	**-**	**1**	**-**	**2**	**positive**	**2**	**+**	**-**	
3	**-**	**3**	**-**	**1**	**+**	**3**	**-**	**3**	**positive**	**1**	**+**	**-**	
4		**1**		**1**		**1**		**5**	**positive**	**1**	**+**	**-**	**+**
5	**+**	**1**	**-**	**1**	**+**	**1**	**+**	**1**	**positive**	**1**	**+**	**-**	
6	**-**	**1**	**-**	**1**	**-**	**1**	**-**	**1**	**positive**	**1**	**+**	**-**	
7	**-**	**1**	**-**	**1**	**-**	**1**	**-**	**1**	**positive**	**1**	**+**	**-**	
8		**2**		**1**		**1**		**1**	**positive**	**1**	**+**	**-**	**+**
9		**2**		**1**		**1**		**2**	**positive**	**1**	**+**	**-**	
10		**1**		**1**		**2**		**2**	**positive**	**1**	**+**	**-**	
11		**1**		**1**		**2**		**1**	**positive**	**1**	**+**	**-**	
12		**1**		**1**		**1**		**1**	**positive**	**1**	**+**	**-**	**+**
13		**1**		**1**		**1**		**1**	**positive**	**1**	**+**	**-**	**+**
14		**1**		**1**		**1**		**1**	**positive**	**1**	**+**	**-**	**+**
15		**1**		**1**		**1**		**1**	**positive**	**2**	**+**	**+**	
16		**1**		**1**		**1**		**1**	**positive**	**1**	**+**	**-**	**+**
17		**4**		**2**		**2**		**3**	negative	**1**	**+**	**+**	
Allergic patients													
AD													
18	+	5	-	1	-	4	-	5	positive	1	+/-	-	
19	+	4	-	1	-	3	-	3	positive	1	+/-	-	
20	+	2	-	1	-	1	-	1	positive	1	+/-	-	
21	-	1	-	1	-	1	-	1	positive	1	+/-	-	
22		1		1		1		1	positive	1	+/-	-	

23	+	4	-	3	+	3	+	4	negative	1	+/-	-	
24	+	4	-	1	+	2	+	3	negative	2	+/-	-	
25	-	4	-	1	+	1	-	2	negative	1	+/-	-	
26	+	2	-	1	+	1	+	3	negative	1	+/-	-	
AFR													
27	-	2	-	1	-	1	-	1	negative	1	+/-	-	
28	-	1	-	1	-	1	-	1	negative	1	+/-	-	
29		1		1		1		1	negative	1	+/-	-	
Negative control group													
30		**4**		**2**		**1**		**3**	**negative**	**1**		**-**	
31		**3**		**1**		**5**		**4**	**negative**	**1**		**-**	
32		**3**		**3**		**3**		**3**	**negative**	**3**		**-**	
33		**2**		**2**		**3**		**3**	**negative**	**4**		**-**	
34		**2**		**1**		**1**		**1**	**negative**	**1**		**-**	
35		**2**		**1**		**2**		**1**	**negative**	**2**		**-**	
36		**3**		**1**		**3**		**3**	**positive**	**1**		**-**	

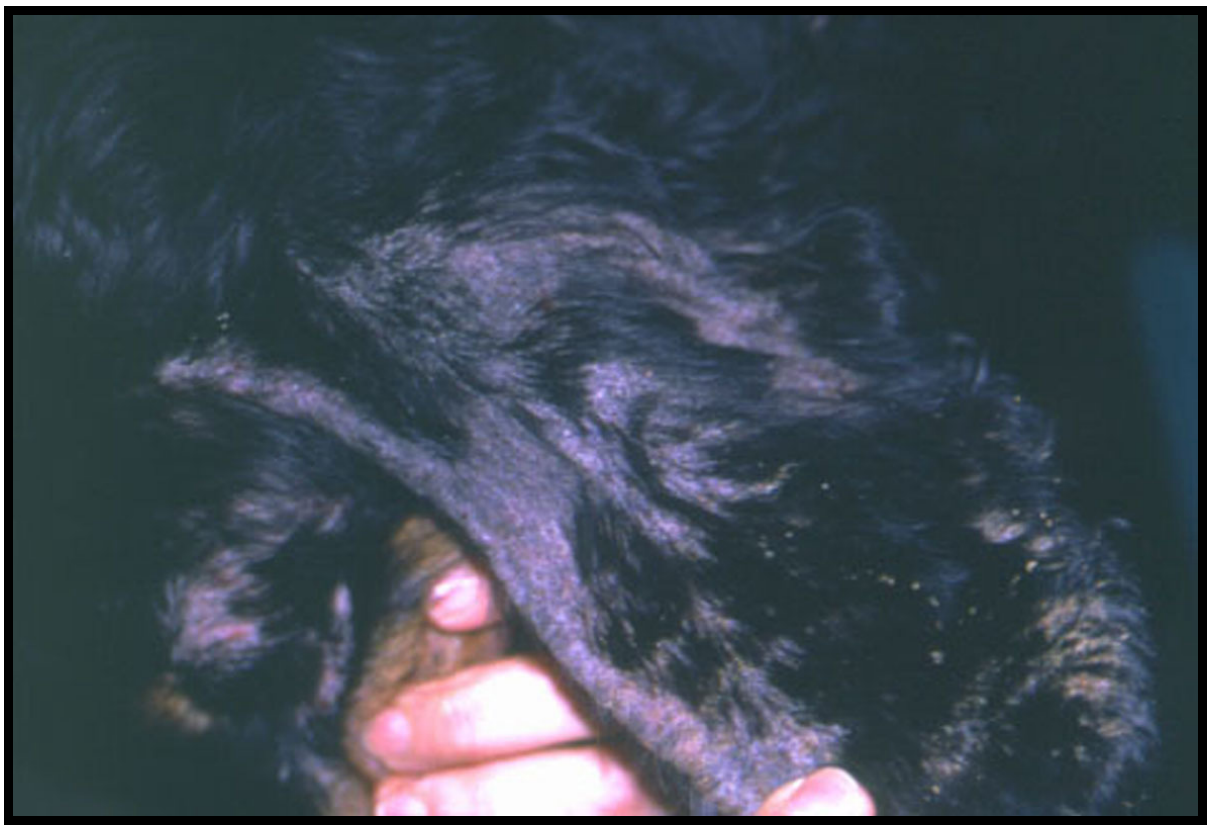


Figure A. The traumatic alopecia, encrustation and crusted papules on the ear margin of a cocker spaniel with *Sarcoptes* infestation (N. Tarpataki).

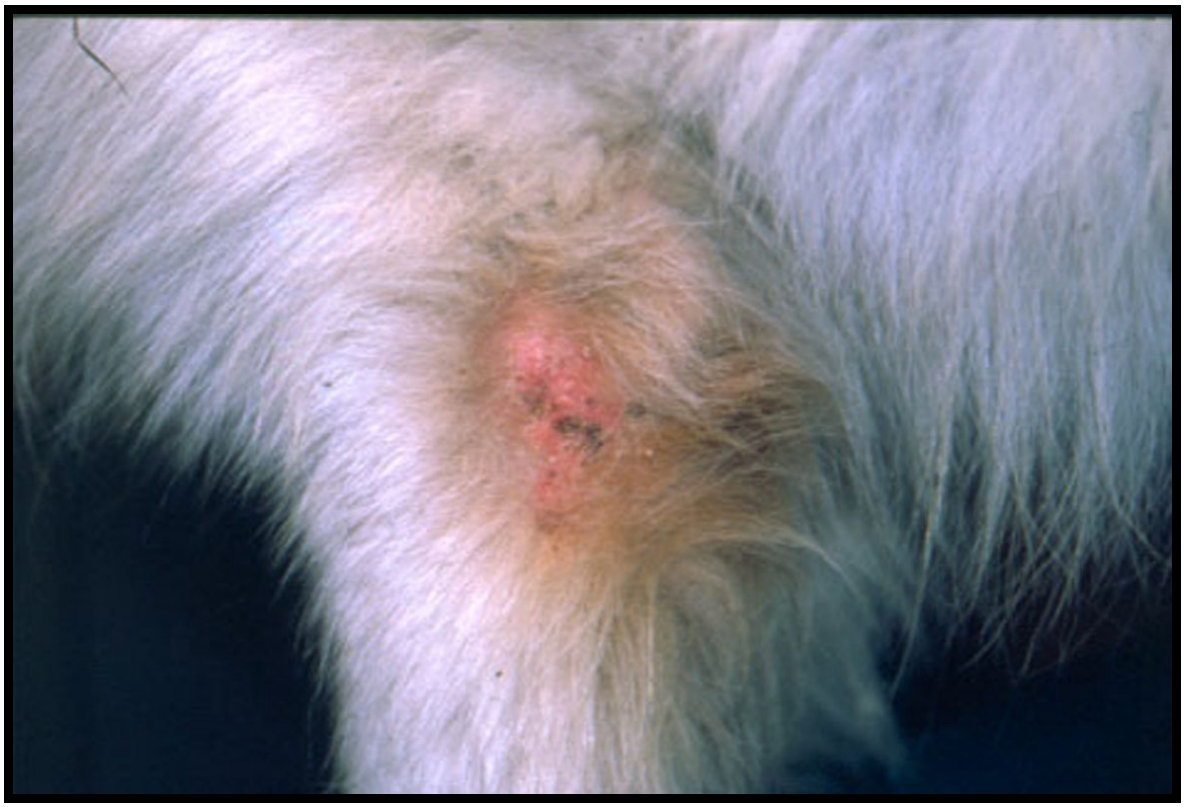


Figure B. The traumatic alopecia, erythema, excoriation and crusted papules on the elbow of a spitz with *Sarcoptes* infestation (N. Tarpataki).

7. Chapter VI

Specific immunotherapy and experiences of its use in Hungary in dogs suffering from atopic dermatitis

Tarpataki N.: A specifikus immunterápia (hyposensibilisatio, desensibilisatio) és hazai alkalmazásának tapasztalatai atópiás bőrgyulladásban szenvedő kutyákban, I. Rész: Irodalmi áttekintés. (*Magyar Állatorvosok Lapja*, 125. 99-108., 2003/2.)

Tarpataki N.: A specifikus immunterápia (hyposensibilisatio, desensibilisatio) és hazai alkalmazásának tapasztalatai atópiás bőrgyulladásban szenvedő kutyákban, II. Rész: A specifikus immunterápia a gyakorlatban (Irodalmi áttekintés) (*Magyar Állatorvosok Lapja*, 126. 403-411., 2004/7.)

Tarpataki N., Bagdi Nóra, Pápa Kinga, Papp László, Vörös Károly: A specifikus immunterápia (hyposensibilisatio, desensibilisatio) és hazai alkalmazásának tapasztalatai atópiás bőrgyulladásban szenvedő kutyákban, III. Rész: A specifikus immunterápia a gyakorlatban (Saját vizsgálatok) (*Magyar Állatorvosok Lapja*, 126. 545-552. 2004/9.)

Tarpataki N.: A specifikus immunterápia és hazai alkalmazásának tapasztalatai atópiás bőrgyulladásban szenvedő kutyákban, I. Rész: Irodalmi áttekintés (*Kisállatpraxis*, 4: (2) 46-54., 2003/2.)

Tarpataki N.: A specifikus immunterápia és hazai alkalmazásának tapasztalatai atópiás bőrgyulladásban szenvedő kutyákban, II. Rész: A specifikus immunterápia a gyakorlatban (Irodalmi áttekintés) (*Kisállatpraxis*, 5. (6.) 19-26., 2004/6.)

Tarpataki N., Bagdi N., Pápa K., Papp L., Vörös K.: A specifikus immunterápia és hazai alkalmazásának tapasztalatai atópiás bőrgyulladásban szenvedő kutyákban, III. Rész: A specifikus immunterápia a gyakorlatban (Saját vizsgálatok) (*Kisállatpraxis*, 2005/1.)

1. Review of literature

There are three modalities for the treatment of allergic conditions: avoidance of exposure to allergens, systematic therapy and immunotherapy (Reedy et al. 1997).

The precise etiological diagnosis is the basis of targeted treatment (allergen elimination and immunotherapy). The ideal solution would be the elimination of allergens from the environment as in the absence of allergens no symptoms develop and no treatment would be needed. However, bearing in mind the wide range of allergens, complete elimination of allergens is only feasible in cases of feed allergies and contact allergies, although it can rarely be managed in case of dermatitis associated with aeroallergens.

Symptomatic therapy is confined to the transitional reduction and elimination of the symptoms. Until this day there is no evidence that the increasingly progressing (see later) atopy could be alleviated by pharmacological therapy. The most striking characteristic of the promise of immunotherapy is that it may lead to lasting improvement (Creticos 1992). In humans immunotherapy by changing the course of pathological processes may prevent the development of sensitivity to other, new allergens (Des Roches 1995).

The aim of the present review is to summarize the theoretical and practical background of specific, targeted immunotherapy (Part 1) and the first therapeutic efforts (Part 2).

Definition of technical terms

In allergology immunotherapy (IT) is understood as *the injection of gradually increasing doses of pathogenic allergens to allergic patients in order to increase the tolerance of the patients and to reduce the sensitivity to pathogenic allergens.* Immunotherapy includes the influencing of the immune system of the patient. As long as the process is efficient the process can be termed as immuno-modulation. The use of terms desensitization and hyposensitization are incorrect in the case of atopy, since these include such processes which are not precisely characteristic of atopy. Although published research is using the three terms as synonimes, the term “immunotherapy” has a more general meaning and its use is preferred to desensitization and hyposensitization (Reedy et al. 1997).

Pathomechanism of immunotherapy

Although there is a vast body of human research data, the mode of action of immunotherapy and its clinical efficacy is far from being completely understood. In immunotherapy the very

first response is the rise of allergen-specific IgG serum antibodies. These IgG antibodies acting as blocking antibodies form immune-complexes binding circulating allergens and preventing allergen induced mast cell degranulation. This very first response occurs only several months after the start of the treatment and this phenomenon explains the “early” response. Serum IgG levels correlate with the applied allergen concentration, but there is no such direct relationship with the efficacy of immunotherapy. In some studies correlation was demonstrated to clinical response (Connel et al. 1970), while others demonstrated no such relationship between IgG levels and clinical response. The determination of IgG levels fails to predict the efficacy of immunotherapy, as the highest IgG levels do not occur in animals with the weakest clinical symptoms. Following an initial rise IgG levels show a plateau which remains stable during immunotherapy.

During the initial introductory phase of immunotherapy the second event is the increase of both circulating and cell-bound allergen specific IgE type antibodies. However, this does not seem to be a pathogenic response and is probably related to the low IgE affinity of the Fc receptor molecules and later the IgE positive reaction shifts to IgE negative one (Malling et al. 1993). Lichtenstein reported in 1988 the heterogeneity of IgE molecules calling IgE positive the molecule interacting with histamine releasing factor (HRF) and IgE negative the rest of IgE antibodies.

In case of long term immunotherapy the allergen specific IgE levels usually decrease, but there are very few human patients where IgE is completely eliminated from serum, which means that no real and complete desensitization occurs. In case of seasonal atopy patients the characteristic post-seasonal IgE rise is not detected indicating the development of a sort of immunological tolerance. Thus modulating effect is considered to be the effect of immuno-therapy on T lymphocytes as these cells regulate antibody production (DeVries 1994). The anti-ragweed IgE levels are closely correlated with the severity of symptoms in untreated atopic patients. However, neither declining IgE levels, nor the rise of allergen specific IgG antibody levels reflects the efficacy of immuno-therapy. According to a survey (Nish et al. 1994) allergen specific IgE antibody level declined only in two out of nine patients six month after the start of the immunotherapy, while allergen specific IgG levels increased in eight cases. Clinical symptoms can be relieved by the decline, maintenance and increase of allergen specific IgE antibody levels as well.

It seems generally accepted that successful immunotherapy is based on T-cell modulation and immunotherapy affects the control mechanism of immune response and not only antibody production. This is supported by the fact that no allergen specific T-lymphocyte suppressor cells were detected in untreated atopic patients, but in case of immunotherapy these cells appear in the blood. In case of poison allergy (eg. insect bite allergy) immunotherapy was demonstrated to reduce the expression of low affinity IgE receptors on the surface of T and B-cells (Prinz et al. 1991). In addition the sensitivity of mast cells is also decreased (Malling et al. 1993) and as result larger antigen "load" is needed and in turn this reduces the release of chemical mediators. Most probably the effect of immunotherapy is uncoupling allergic reaction by disrupting the chain of events characteristic of the allergic conditions. The essence of the process can be summarized as follows.

1. At first it induces T-helper type 2 effector cells to differentiate to T-helper 1 cell. This way IL-2 and IFN-gamma responding citokine profile primarily results that IL-4.dependent IgE production is inhibited. This process is amplified by reduction in the number of T-helper 2 cells which in turn reduces IL-4 production.

2. It also reduces the activity of mast cells by three ways. Local IgE production is reduced, IL-3 dependent activation is missing and histamine releasing factor production is reduced.

Along with these processes the mast cell cytokine independent mediator release may occur as well as the shift from IgE-positive overload is being switched to negative. Along with the reduced IL-5 production the activity of eosinophil cells is also reduced thus leading to reduced inflammation and tissue destruction.

Is is known that an allergic reaction is a two-phase reaction, it is worth to demonstrate the effect of immunotherapy. Following the two phase degranulation (early response) a second response occurs occurs which is called late phase reaction (LPR). This late response is characterized by neutrophil and eosinophil granulocytes (Georgitis 1995). In the late phase further leukotriene and histamine release develops, indicating that potentially in addition to mast cells other mediators also possess inflammatory mediators. During the late phase reaction tissues respond to lower staring allergen doses and are more sensitive (Becker et al. 1989). According Iliopoulos immunotherapy is reducing the late phase response of the skin much more than the immediate response, but he could not provide explanation for precise mechanism (Iliopoulos 1991). The allergen induced reduced HRF production is one of the explanations for the reduced histamine production in the late skin phase. Walker (Walker et al. 1995) assumed that in case of immunotherapy the reduction of the immediate skin response may occur as a result of the reduction in the number of mast cells and the late phase response suppression results in the modification of response of the T-lymphocytes to

allergens. In the immunotherapy no significant correlation was found between the level of the of the change in late phase and the seasonal symptoms and the requirement for pharmaceuticals, but the reduction of the late phase reaction may represent the early clinical symptoms. The simultaneous effects of immunotherapy on different lymphokines and cytokines directly affect cellular response. This assumption is supported by the fact that immunotherapy changes allergic inflammatory response through T-cell tolerance (VanMetre 1993).

Immunotherapy significantly reduces both immediate and late phase histamine release (Kung et al. 1989). On this ground it is assumed that immunotherapy exerts "down-regulating" effect on both processes, ie. by the inhibition of both mast cells (early release of histamines) and basophyl granulocytes (late histamine release) thus preventing pathological events. Most probably immunotherapy rather reduces T-helper 2 cell functions and its cytokine production and not by exerting quantitative effects on inflammatory cells other than lymphocytes. Immunotherapy is able to reduce HRF and to amplify the production of its inhibitory factor (IL-8) (Hsieh 1995). This process explains in part the clinical efficacy of immunotherapy. Immunotherapy reduces lymphocyte proliferation in response to allergens and the production of citokines (mitogen factor, macrophag inhibitory factor) (Evans et al. 1976). It is assumed that efficacy of immunotherapy is attributed to more than one simultaneous effect including reduced specific and non-specific cellular response, antibody production and changes at cellular levels (VanMetre et al. 1993).

Allergen immunotherapy in dogs

Allergen immunotherapy has been widely used in atopic dermatitis of dogs for several years. In contrast to human atopic dermatitis clinical results are promising. In 60 per cent of the cases partial recovery was attained by hyposensitation (Nuttal and Thoday 1998; Willemse 1994). It is very difficult to explain the difference between human and canine atopic dermatitis immunotherapy treatment results. It may occur that interpretation of clinical settings explain the difference: in case of canines success it is more easily attributed to clinical improvement. Unfortunately, there is no way to compare human and dog studies due to the lack of standardized allergen solutions, treatment protocols and definition of success in treatment. For the same reason the results of dog treatments are also hardly comparable. The difference in the efficacy of immunotherapy between humans and dogs can be that in dogs type I. hypersensitivity response may play a much important role in the pathogenesis of atopic dermatitis. The observation that the efficacy of immunotherapy in dogs is negatively influenced by the longer duration of atopic dermatitis (Nuttal and Thoday 1998) suggests that in a similar way to human patients specific immunotherapy exerts is benefits during the acute phase skin symptoms.

Therapeutic mixtures and their use

There are three types of therapeutic mixtures in practical use.

1. The water soluble allergen is absorbed immediately, lower doses are needed, but application has to be frequent. This is the most popular form used overseas and this results in the fastest hyposensitization (Scott et al. 2001). Several treatment regimes are in use and one of them is shown in the **Table 1**.

Table 1. Proposed protocols to water allergen solutions (Scott et al. 2001)

No.of treatment	Day	1st solution (100-200 PNU/ml)	2nd solution (1000-2000 PNU/ml)	3rd solution (10000-20000 PNU/ml)
1.	1	0,1 ml		
2.	2	0,2 ml		
3.	4	0,4 ml		
4.	6	0,8 ml		
5.	8	1,0 ml		
6.	10		0,1 ml	
7.	12		0,2 ml	
8.	14		0,4 ml	
9.	16		0,8 ml	
10.	18		1,0 ml	
11.	20			0,1 ml
12.	22			0,2 ml
13.	24			0,4 ml
14.	26			0,8 ml
15.	28			1,0 ml
16.	38			1,0 ml
17.	48			1,0 ml
18...	at 20-40 day intervals			1,0 ml

There is an emergency or accelerated protocol which attains the maximal 20,000 PNU/ml antigen concentration within 6 hours. In trial the method was tested in 6 experimental animals and 14 clinical cases and neither side effects nor clinical efficacy reduction was observed (McDonald 1999). Accelerated hyposensitization is a crucial procedure in life threatening conditions, eg. insect-bite allergies (Reedy et al. 1997).

2. Aluminium-hydroxid absorbed allergens fall between aqueous and emulsion preparations regarding their duration of effect (Wagner 1998). Compared to aqueous allergens their absorption is much slower which means that they should be applied in much higher doses and less frequently. Their disadvantage is being aluminium precipitates and may be potentially carcinogen and their use is diminishing in the USA. Despite this fact, in Europe these are the most widely used preparations (Prélaud 1991; Walkers et al. 1995; Willemse et al. 1984; Willemse 1994).

3. Allergen emulsions are mixtures of allergens and propylene-glycol, glycerol or other mineral oils. They are slowly absorbed, therefore these are applied with the largest intervals and the largest doses. Systemic side effects are minimal, but their use is shrinking as in mice their application was accompanied by myeloma and sarcoma (Scott et al. 2001).

Optimal timing to start immunotherapy

In case of seasonal allergy, out of the "co-seasonal, pre-seasonal and post-seasonal" applications the best results are expected in post-seasonal treatment as the time needed to reach full efficacy is 3-4 month. If therapy is started following the season by the time the next season starts the maintenance therapy will be reached. In case of non-seasonal atopy therapy can be started at any time of the year.

Number of simultaneously administered allergens

Hyposensitization with more than 10 allergens was previously discouraged (Reedy et al. 1997, Scott et al. 2001). In recent years, however, solutions containing 30 (!) allergens are also used. According to a study hyposensitization using more than 30 water suspended allergens was safe and successful (Scott et al. 2001). In a retrospective study response to treatment was observed in 72% of cases treated with 1-10 allergens, 86% of cases treated with 11-20 allergens and 78% in cases treated with 21-40 allergens (Angarano and McDonald 1991). In two other studies no relationship was found between treatment success rate and number of allergens in the mixture used for treatment (Nuttal and Thoday 1998, Scott and Rosychuk 1999). For those atopic dogs which demonstrate response to several antigens (positivity to 0-30 or more allergens) allergens should be selected according the probability of prevalence in the environment.

Expected onset of clinical improvement

There is a great variability in the onset of clinical improvement in response to immunotherapy. The timing depends on the severity of the allergy and the immunoresponsiveness of the patient. Depending on the form of allergen applied and the individual animal, improvement may start already in the second week of the treatment, but favorable response was also observed 18 (!) month after the commencement of the immunotherapy. In a study 41% of the treated animals responded in the first 2 month of the therapy and 71% of the patients showed improvement within six month. If an atopic patient tends to respond to treatment then it occurs during the first 9-12 month of the treatment (Walker et al. 1995). According to other reports the lack of success of immunotherapy can be established already in 6 (Kunkle 1980) and 8-9 month (DeBoer 1989) as long as no improvement is observed. Out of the responders 25% responds in the first three month, 50% in 3-6 month and another 25% after 6-12 month (Reedy et al. 1997).

Recurrence of symptoms during immunotherapy

Sudden or serious symptoms may hint on ectoparasites (flea, mange) or secondary (bacterial and/or fungal) super-infections and these should be excluded. The symptoms of flare ups by inhalation allergens are usually more gradual and in most of the cases are associated with the uptake of large volumes of pollens. In most of the cases a short symptomatic therapy leads to improvement. Relapse may occur due to sensitization to a new allergen, especially if the patient responded favourably to immunotherapy during the preceding one year. If the symptoms are serious the possibility of other allergic diseases (feed allergy, contact allergy) should be considered.

Other factors affecting efficacy of immunotherapy

Regarding the response to immunotherapy no sex or breed difference was observed. One author (Willemse 1994) mentions that boxers and West Highland terriers were less sensitive to hyposensitization. In contrast to the previous observation (Scott 1981) that those dogs which are treated with smaller number of allergens (2-10) show better response than those treated with 12-20 allergens, today it seems that there is no such difference in function of the number of allergens used in the solute (Angarano and McDonald 1991; Scott et al. 1992). According to surveys dogs allergic to pollens generally respond better to hyposensitization (81%) than those allergic to other allergens (59%). Immunotherapy with individual pollens seems to be more successful than immunotherapy with groups of pollens (Reedy et al. 1997). According to another study immunotherapy patients showing strong response to pollens 57,6%, house dust 44%, yeast 12,5% and in insect allergy 29,6% responded favorably, respectively. Among those showing weak response only 32% responded favorably (Mueller 1995). According to some studies dogs older than 8 years respond

less favorably to hyposensitization than younger ones (Austin 1976; Willemse 1994). Dogs treated within three years of the onset of the allergy showed a better response rate than those treated later (Mueller 1995). Those tested within one year showed 71% response, those tested between one and three year showed 60% response while those suffering allergy for more than five years showed 57% response to immunotherapy. Others claim that age has no influence on therapy (Scott et al. 2001). Success can also be influenced by the maintenance dose rate. In case of high level specific allergen (40,000 PNU/ml) the treatment of 133 dogs reduced symptoms to half of the previous level in 85% of the cases in contrast to those with lower dose rate (10,000 PNU/ml), where similar improvement was seen only in 68% of the dogs (Reedy et al. 1997).

Long term immunotherapy

Until this day no report was published on the possible side effects of long term immunotherapy (Reedy et al. 1997, Scott et al. 2001). In human studies, as well, years long immunotherapy surveys failed to demonstrate collagen, autoimmune or lymphoproliferative disorders as side effects (Malling 1995). The efficacy of immunotherapy should be evaluated at least annually and according to the result decision should be taken regarding the continuation of the immunotherapy. The average treatment duration in humans is 3-5 years depending on the seasonal or environmental nature of the allergen (Patterson 1983). In case of pollen allergy the duration of the protection may be 6 years following the termination of the treatment (Walker et al. 1995), while in dust mite allergy it is even questionable whether or not treatment can be discontinued. The shorter the duration of the therapy the more probable is the recurrence of the disease (McDonald 1999) which can be of different magnitude. Minimal symptoms may occur which can be eliminated by the control of the environment or occasional symptomatic therapy, but in more severe cases the therapy should be re-started. According to a study only in 10% of the atopic dogs could the therapy be discontinued on the average after 2 years of treatment (Willemse 1994). Similar results were obtained in the survey of Baker (1990). It is a general procedure for dermatologists to discontinue maintenance treatment after one symptomless year. In dogs experience have shown that in most cases relapse occured within one year of discontinuing the treatment (Nacleiro 1995). Therefore a life long maintenance therapy is recommended.

Immunotherapy is a very slow, time consuming and expensive treatment option. On the top of it the success or failure of hyposensitization is based on the satisfaction or dissatisfaction of the owner. The better the owner is educated in the disease process the higher the chance of his/her satisfaction even if recovery is not complete.

2. Observations on specific immunotherapy in dogs with atopic dermatitis

Materials and methods

In the present study the efficacy of specific immunotherapy on 20 atopic dogs at the dermatology clinic of our Department. In the 20 atopic dogs the pathogen allergens were detected with 'Artruvetrin Test Set' allergen test solutions of ARTU Biologicals, The Netherlands (**Figure 1-2**). Those 25 allergens were used which have the highest prevalence in Hungary as established with histamine (positive) and phosphate-buffer (negative) controls. Hyposensitization was done with the following allergens: dust mite (*D. farinae, D. pteronyssinus, A. siro, T. putrescentiae, L. destructor*) molds (*Penicillium notatum, P. notatum, Trichophyton mentagrophytes, T. mentagrophytes*), feline epithelial cells, human epithelial cells, feather (hen, goose), ragweed, dandelion, timothy grass, orchardgrass, willow, oak. The allergens which elicited response were used for the formulation of an aluminium-hydroxide absorbed allergen mixture by the manufacturer in the preparation called 'Aruvethrin Therapy' (**Figure 3**) according the results of the preliminary individual diagnosis. The solution used for hyposensitization was used according to the protocol shown in T**able 2**.

Table 2. Recommended protocol to Artuvetrin Therapy Preparation

No. of Treatment	Timing	Volume of sc. injection solution (ml)
1.	1st day	0,2
2.	2nd week (day 15)	0,4
3.	4th week (day 29)	0,6
4.	6th week (day 43)	0,8
5.	9th week (day 64)	1,0
6.	12th week (day 85)	1,0
7.	16th week (day 113)	1,0
8.	20th week (day 141)	1,0
9.	24th week	1,0
10.	28th week	1,0
11.	32nd week	1,0
12.	36th week	1,0

The concentrations for the different allergens were the follow: mites (25-100 NU), pollens (250-1000 NU), insects (25-100 NU), epithelia (25-100 µg), fungi (25-100 µg), materials (2.5-10 µg). If

a dog received one mite the concentration for the mite was 100 NU/ml, for 2 different mites the concentrations for the different mites were 50-50 NU/ml, and for 3 mites these were 33-33 NU/ml.

For the evaluation of efficacy each owner with at least one completed treatment cycle was presented with a questionnaire with multiple choice and comments were also collected. The following questions and replies were recorded:

1. Are you overall satisfied with the efficacy of the serial injections? (Y/N)
2. Would you recommend the treatment to other owners with similar problems? (Y/N)
3. How efficient do you find hyposensitization on the basis of percentual reduction in itching, reduction of skin symptoms provided the starting condition is scored as 100 per cent? (reduction to 10-20 per cent, very much; to 30-40 per cent, substantially; to 50 per cent, to half; to 70-80 per cent, slightly; to 90-99 per cent, hardly, did not reduce symptoms).
4. When did the effect of the treatment start to become visible, on which subsequent injection started the symptoms subdue? (1^{st} day, 2^{nd} week, 4^{th} week, 6^{th} week, 9^{th} week, 12^{th} week, 16^{th} week, 20^{th} week, 24^{th} week, 28^{th} week, 32^{nd} week, 36^{th} week).
5. During hyposensitization was there a need for other treatments and if so, which preparations were used? (Y/N, ...).
6. Was there any deterioration of the condition (itching, skin symptoms) within two days immediately following the administration of any injections? (Y/N).
7. Was there any sudden (anaphylactic) symptom immediately within half an hour following any injection administration (Y/N).
8. How many injection cycles have been administered and will/have you order/ed new solution and if so which will be the cycle number of the new solution? (N/Y,....).

The survey was supported by IAMS by rewarding the owners completing the questionnaire with EUKANUBA Dermatosis FP response Formula make dry feed.

Results

The key anamnesis data and responses to the questionnaire are shown in **Table 3.** The dogs belonged to 5 breed groups (five mixed and one each breed), including 11 males and 9 bitches, ranging from 9 months to 7 years of age. The symptoms were present in 50% of the cases for shorter than 1 year, in 30% for 1-2 year, and longer in the remaining 4 cases. Symptoms appeared on the average at 17 months of age and lasted for 19,3 months. In case of 19 dogs (95%) the first cycle was already completed and with one dog the cycle was interrupted. In seven cases (35%) symptoms were seasonal including two cases where seasonal symptoms turned permanent and in 13 cases (65%) non-seasonal skin complaints (itching) was present. In one dog serous nasal discharge was the predominant symptom.

In responding to the questionnaire 14 owners (70%) were satisfied with the effect of immunotherapy and six owners (30%) were dissatisfied including two who would not recommend the treatment to other owners with similar problems.

Eleven owners (55%) have already placed orders for the 2nd, 3rd and 4th injection cycles. To the question how effective they found hyposensitization four owners replied that the improvement was 80% or above, two reported substantial (60-80%) improvement. In four cases (20%) symptom intensity declined to 40-60% and in 6 dogs only a slight improvement (20-40%) was observed. A minor improvement (10-20%) was seen in 3 cases (15%) and in one case (5%) no improvement was seen (**Figure 4**).

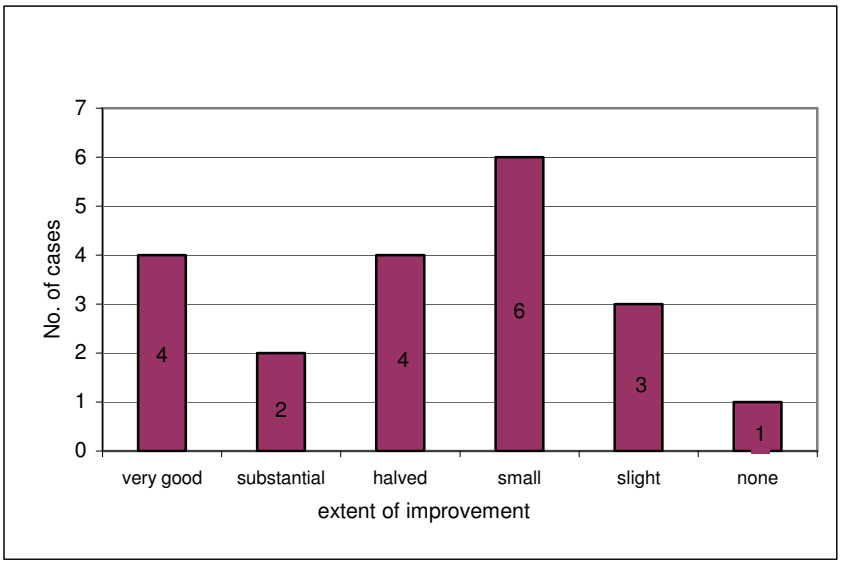


Figure 4. Distribution of effectiveness of immunotherapy.

Excluding this latter patient all the others were posed the question: when was the sign of first improvement observed. The earliest observations were made at the 4th injection (at 6 weeks) in 4 dogs (21%), while in three (16%) at 9 weeks, and in three (16%) at 12 weeks, respectively. Four other owners reported improvement at the 16th week (7th injection) and one owner at 24 weeks (9th injection). Three owners (16%) reported effect only at week 28. In one dog with seasonal symptoms pollen season started at the 32nd week of treatment, but seasonal symptoms appeared only 6 weeks later, meaning that the onset of the symptoms were postponed by 6 weeks into the season by the serial injections started immediately after the preceding season (**Figure 5**).

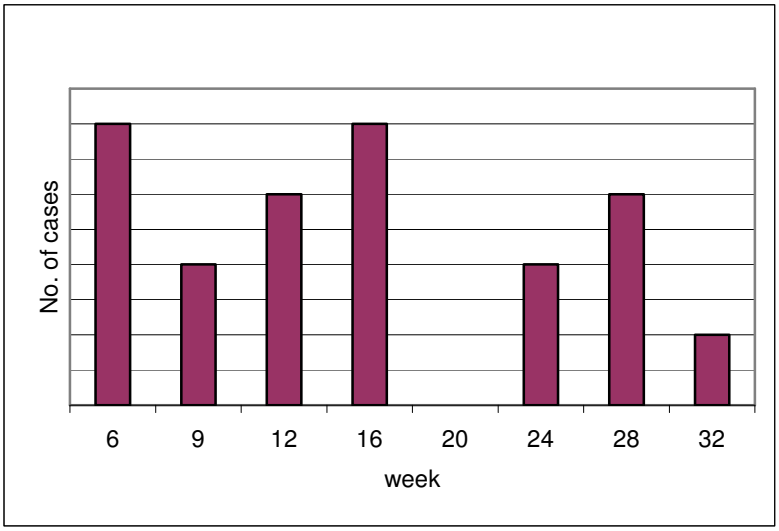


Figure 5. Beginning of effect of immunotherapy from the first treatment.

In the six patients where symptoms alleviated or substantially the average age was 12,3 month with an average duration of symptoms of 9,2 months. In the four patients where treatment was slightly or hardly effective the average age was 15 months and average duration of symptoms was 19,5 months (**Figure 6**).

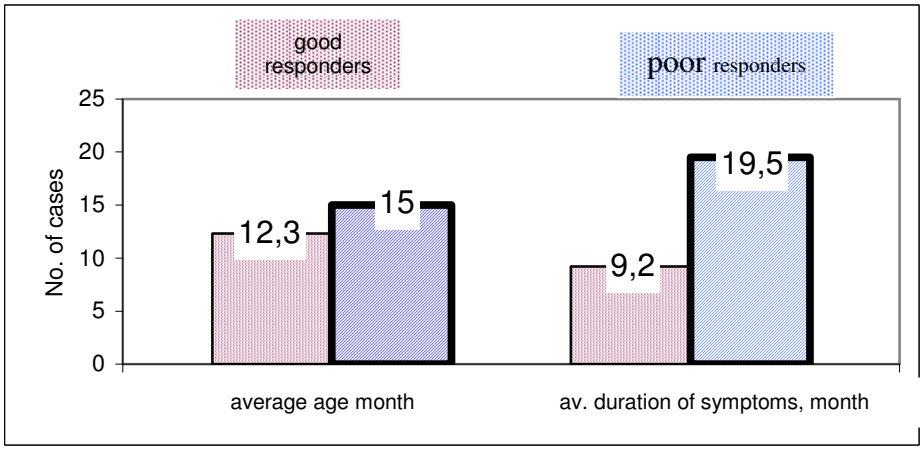


Figure 6. Average age and average time of existence of symptoms in the groups of patients reacting to the therapy significantly or well (first columns) and not or poorly reacting (second columns).

Permanent and reliable flea control was requested from each owner as basic precondition. The distribution of supportive treatment in function of severity of symptoms was as follows. No supportive therapy was needed in 7 cases (30%). In the rest of the cases, however, other modalities are also administered. In most of the cases (7 dogs, 35%) the use of some anti-histamines was suggested (Atarax tabl. (hydroxizin), Fenistil 24 capsule (dimentinden-maleate), Suprastin tabl. (chlorpyramine), Tavegyl tabl. (clemsatin), Claritine syrup (loratadin) at least in the early phase of the treatment. Medicated shampoo (Dermilene A.U.V, Peroxyderm A.U.V. or Lactaderm A.U.V) was recommended in 7 cases (35%) and local treatment was needed in 4 cases (20%) with other anti-inflammatory substances (Penochron-N emulsion A.U.V., Pruritex solution A.U.V., etc.). Due to secondary bacterial skin infection two dogs (10%) were treated with systemic antibiotics (Aktil tabl., Pyassan capsule). In one case (5%) the symptoms could not be controlled without the administration of glucocorticoid (Prednisolon tabl.).

The possibility of side effects upon the administration of the therapeutic solution was also subject of questioning. The deterioration of the starting symptoms within two days following treatments occurred in 4 cases (20%). In no case was local or systemic anaphylactic reaction observed. Of the four owners whose dog currently participates in the fourth or fifth cycle none mentioned any side effects.

Under the heading “other remarks” of the questionnaire the followings were recorded: one patient following the second cycle required supportive anti-histamine treatment only during the first cycle. The only patient with nasal discharge and respiratory symptoms, which responded to dust mite (*D.farinae*), meal mite (*A. siro*) and cat epithelial cells received the maintenance dose at 3 weeks intervals for the sake of symptomless condition. (This dog was under hyposensitization for four consecutive years).

The other satisfied owner’s dog also participated in hyposensitization and its Vetaraxoid treatment, which was continued since 5 months of age, could be discontinued only during hyposensitization and this resulted in the elimination of the symptoms. Most of the remarks referred to the reduction of the intensity of itching but not its complete elimination. The reduction in itching intensity reached a level where secondary skin problems (secondary skin pruritus, emaceration, Malassezia-dermatitis) did not provoke itching. In patients with seasonal skin problems the onset of the season was postponed by 2-6 weeks, but owners were not completely satisfied with the effect. Two owners were dissatisfied with the reduction of symptoms to half of the previous level and one of them would not even recommend the treatment to others. Others (4 owners) were satisfied with a lesser improvement of symptoms indicated by their enrollment for the 3rd, 4th or 5th treatment cycle.

Table 3. Anamnesis, breed, results of examinations and treatment efficacy in 20 dogs treated with SIT.

No.	Breed	Age at onset of symptoms	Duration of symptom	Sex	Symptoms, seasonality	Pathogenic allergens	Satisfaction	Would you recommend?
1.	Mix	2,5 yr	1,5 yr	M	aseasonal, pruritus	Derm.far. Pen. not.	Y	Y
2.	Shar-pei	3 mo	3 mo	M	seasonal pruritus	Derm.far. Derm.pter. Acarus s. ragweed	Y	Y
3.	Bobtail	2 yr	2 yr	M	aseasonal pruritus, pyoderma	Derm.far. Acarus s. human epithel, cat epithel, hen feather	Y	Y
4.	Spaniel	3 yr	3 mo	M	aseasonal pruritus	Derm.pter. Acarus s. Lep. destr.	Y	Y
5.	Boxer	1 yr	2 yr	M	aseasonal pruritus	Derm.far. human epithel	Y	Y
6.	Akita	2 yr	7 mo	F	aseasonal pruritus	Derm.far. Acarus s. Tyr.putr.	Y	Y
7.	Mix	3 yr	4 mo	F	aseasonal pruritus	Derm.far. Acarus s. Tyr.putr. Trich ment.	Y	Y
8.	Chow-Chow	8 mo	7 yr	F	seasonal pruritus turned aseasonal	Derm.far. Acarus s. Tyr.putr. wormwood, ragweed, willow	N	Y
9.	Mix	1 yr	6 yr	M	seasonal pruritus turnd aseasonal	Derm.far. Acarus s. Lep.destr.	Y	Y
10.	Chow-Chow	1 yr	3 yr	M	aseasonal pruritus	Derm.far. Acarus s.	N	Y
11.	Leonber.	6 mo	1 yr	M	bilateral nasal discharge	Derm.pter. Acarus s. cat epithel	Y	Y
12.	dalmatian	1 yr	3 yr	F	seasonal pruritus	Derm.far. timothy, common wormwood	Y	Y
13.	Fila brasilero	2 yr	8 mo	F	aseasonal pruritus	Derm.far. Acarus s. Tyr.putr. oak	Y	Y
14.	Doberma nn	2 yr	1 mo	F	aseasonal pruritus	Derm.far.Derm.pter. common wormweed	N	Y
15.	Mix	6 mo	4 mo	M	seasonal pruritus	common wormweed, ragweed	Y	Y
16.	Gordon setter	2 yr	9 mo	M	seasonal pruritus	Derm.far. Acarus s. Tyr.putr. common wormweed, ragweed	N	N
17.	Transylva nian setter	5 mo	14 mo	F	aseasonal pruritus	Derm.far. Tyr.putr. Lep.destr.	Y	Y
18.	English setter	1 yr	6 mo	M	aseasonal pruritus	Derm.far. dandelion, timothy	N	Y
19.	Mix	1 yr	2 mo	F	seasonal pruritus	common wormweed, ragweed, orchardgrass,	N	N

dandelion

20. Westi 6 mo 8 mo F aseasonal prur. Derm.far. Derm.pter. Y Y

Extent of complaint reduction	Time of onset of improvement	Which supportive therapy was used?	Increased itching after injection?	Fainting immediately after injection?	Number of total cycles
Substantial	week 6	Y, local	N	N	2
Substantial	week 9	N	Y, otitis	N	3
Halved	week 28	Y, antibiotic, shampoo	N	N	1
Small	week 6	Y, antihistamine	N	N	1
Small	week 24	N	Y	N	4
Small	week 32	Y, antihistamine, shampoo	N	N	1
Halved	week 6	N	N	N	3
Small	week 16	N	Y	N	1
Small	week 28	Y, antihistamine, antibiotic, shampoo	N	N	5
Halved	week 6	Y, shampoo	N	N	1
Small	week 9	N	N	N	4
Slight	week 16	Y, antihistamine, glucocortic. shampoo	N	N	3
Very good	week 12	Y, shampoo, local	N	N	2
Slight	week 12	Y, antihistamine, shampoo	N	N	1
Very good	week 9	N	N	N	2
Halved	week 28	Y, shampoo, local	N	N	1
Substantial	week 16	Y, antihistamine	N	N	2
None	-	N	N	N	1
Slight	week 16	Y, antihistamine	Y	N	1
Substantial	week 12	Y, local	N	N	4

Discussion

No steps were taken so far as to standardize the "time schedule" or scientifically compare the efficacy of hyposensitization in dogs, although some points were published regarding the form of the applied allergen, its volume, potency, and form of application in injection. In practice the manufacturer of the solution encloses an instruction of use label to the preparation (Scott et al. 2001). As we stayed with the use of the same solution there was no way to come to an conclusion on solution concentrations and treatment schedules. The relatively low number of patients does not allow for conclusions regarding the effect of breed, sex and age. However, it was interesting that age (12,3 months) and duration of symptoms (9,2 months) was lower in the good responding group than in those not or slightly responding to treatment (15 months and 19,5 months, respectively). Especially great efficacy difference was seen in the duration of symptoms (9,2 and 19,5 months) which supports published data (Nuttal and Thoday 1998) that early diagnosis and immunotherapy is more efficient than treatment started at a later stage (Reedy et al. 1997, Scott et al. 2001). Immunotherapy treatment for atopic dermatitis was introduced four years ago in our clinic and for this reason the long term development of protocols, side effects and resumption of symptoms was not studied. Until the present study was completed four patients were already in the fourth year of treatment without any side effects. The present data supported published data (Scott and Rosychuk 1999). 70% of the owners were satisfied with the outcome of the therapy. Therapy reduced symptoms to at least 50% in 11 dogs (55%). In 70% of the patients supportive local or systemic therapies were needed which is in agreement with published data (65%) (Scott and Rosychuk 1999). The onset of effects also showed agreement with published data (Reedy et al. 1997) and in case of non-seasonal symptoms improvement started 3-6 month following the start of the treatment. There were two "peaks" in the onset of improvement as 20% showed improvement at week 6 and another 20% improved at week 16. The difficulty of evaluating the efficacy of the therapy and the associated subjective elements is illustrated by the fact that some owners were satisfied with slight improvement, while others discontinued therapy despite good results going as far as not recommending the therapy to other owners.

In conclusion, the application of immunotherapy poses liability on the veterinarian to alleviate symptoms to the maximum extent and to make the owner to accept the improvement. The veterinarian should decide whether or not the dog is suitable for immunotherapy. The owner should be informed on the treatment of atopic dermatitis treatment including immunotherapy, its benefits and risks, the expected effects, and the occasional supportive treatments. As for research the standardization of solutions, treatment protocols and efficacy determination would be required along with surveys on the results of immunotherapy.

Figure 1. Artuvetrin® Test Set

Figure 2. Artuvetrin® Single Test

Figure 3. The new packaging design for Artuvetrin® Therapy

(www.artuvetrin.com)