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 when 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 incidence nor prevalence of atopic dermatitis in 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 grossly was estimated to be 15% (Chamberlain 1974). In a recent study 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 part of my thesis was to summarize recent developements in canine atopic dermatitis reported duing 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.

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 early work on pathophysiology of 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 accurate 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.

Materials and Methods

In *Chapter II* 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 4 weeks and 10 days, respectively. Calculation of statistical significance was based on Pearson correlation analysis and/or chi-square test by SPSS Version 12.0 for Windows.

In *Chapter III* the author 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 Szent István University, Faculty of Veterinary Science, Department and Clinic of Internal Medicine 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 Pharmazeutische Produkte AG (Muttenz) 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. 23 ARTUVETRIN test allergens were used for IDT but we analysed the results of 8 allergens which were: *Acarus siro (A.siro)*, *Dermatophagoides farinae (D. farinae)*, *Dermatophagoides pteronyssinus (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 filled in cross tables. 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.

In *Chapter IV* 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 and intradermal skin tests were performed in 212 (62+150) pruritic dogs with a clinical diagnosis of atopic dermatitis and 19 (7+12) clinically healthy dogs. All of them were admitted to the Department and Clinic of Internal Medicine, Faculty of Veterinary Science Budapest, Szent Istvan University. Prior to skin testing all anti-inflammatory drug therapy, including oral and topical glucocorticoids and anti-histamines, were discontinued for at least 4 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 culturing the patient was treated by the appropiate antibiotic or antimicotic drug for minimum 3 weeks before sampling. For excluding the Sarcoptes-infestation scabicidal therapy (selamectin (Stronghold® spot on [4]) as spot on solution for systemic treatment (twice in 4 weeks interval)), was prescribed in all (212) patients. All patients (212) had to have a 8 week elimination monodiet to exclude adverse food reaction, 200 dogs had had it before the examination and 12 patients had it after the examination. 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 carbonhydrate (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. 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 to be evaluated. Labor Laupeneck increased the sensitivity of serological test in the meantime. They were then tested allergen-specific IgE specific for house dust mites (*D. farinae*, *D. pteronyssinus*), meal mite (*A. siro*), copra mite (*Tyrophagus putrescentiae, T. putrescentiae*), hay mite (*Lepidoglyphus destructor, 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 . Sera were analyzed using indirect ELISA (imovet biocheck[3]) containing the D9 monoclonal antidbody against canine IgE. The allergen set for intradermal skin testing (Artuvetrin Test solutions) contained the same allergens except 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 was 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 was considered as true positive reactions (TP) and negative results as false negative (FN). Other 12 pruritic dogs were on a 8 week long elimination monodiet only after 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. Respectively, these patients were pruritic, **non-atopic dogs but positive for adverse food reaction (AFR)**. The positive test reactions of this group was 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 min. 2 months. They were tested by the IDT and their sera were tested for allergen-specific IgE too. Positive test reactions of the negative control group was considered as false positive (FP) reactions and the negative test results as true negative (TN) reactions. During evaluation 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).

In *Chapter V* after dermatological examination, blood sera and skin scrapings were taken from 36 dogs. Of the 36, 29 were pruritic, with a tentative diagnosis of *Sarcoptes scabiei* infestation. All the patients (29) were referred by vets to the dermatology unit of the Department and Clinic of Internal Medicine, Faculty of Veterinary Science, Szent István University, Budapest 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 4/5, 3/5, 2/5 were evaluated as intensive, medium and mild pruritus, respectively. 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 was present clinically and in bacterial and fungal culturing, the patient was treated with an appropriate antibiotic or antimicotic drug for at least 3 weeks. All anti-inflammatory drug therapies are 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. 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). Sera were analyzed using 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 (Artu Biologicals), 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) for local treatment (once or twice a week for 4-6 weeks), or selamectin (Stronghold® spot on; Pfizer Inc. Animal Health) for systemic treatment (twice with a 4 week interval), was prescribed and evaluated after two months 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 on 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 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. 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 healthy negative control group (7 dogs), and together with the allergic patients, too, (Bornstein et al. 1996), included all the dogs not responding to acaricidal therapy.

In *Chapter VI* study the efficacy of specific immunotherapy on 20 atopic dogs at the dermatology clinic of the Department of Internal Medicine of the Szent István University's Faculty of Veterinary Medicine. In the 20 atopic dogs the pathogen allergens were detected with

‘Artruvetrin Test Set’ allergen test solutions of ARTU Biologicals, Holland. Those 25 allergens were used which has the highest prevalence in Hungary as established with histamine (positive) and phosphate-buffer (negative) controls. Hyposensitization was effective with the following allergens: dust mite (*D. farinae, D. pteronyssinus, A. siro, T. putrescentiae, L. destructor*) yeasts (*P. notatum, 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’ according the results of the preliminary individual diagnosis.

Results

In *Chapter II* in 66,6% of dogs, the age of onset of atopic dermatitis was, between 4 months and 3 years of age. Dogs living in the garden suburb of Budapest were more sensitive to house dust mites, fleas and molds, and dogs from the western part of Hungary were more sensitive to weeds than to other allergens ($p<0.01$). Positive reactions were most common to *D. farinae* followed by human dander. The breed distribution found in the present study was consistent with that reported in the literature, except for the breeds Hungarian Vizsla, Pumi, French Bulldog, Dobermann Pinscher and Bobtail which were over-represented among atopic dogs compared to the breed distribution of the general dog population of a large city in Hungary. Breeds with verified adverse reaction to food were Cocker Spaniels, French Bulldogs, Bullmastiffs, Bull terriers, St. Bernards, Tervurens, West Highland white terriers and American Staffordshire terriers ($p<0.05$). The clinical signs of atopic dermatitis and their occurence are in accordance with the data described in the literature.

In *Chapter III* results of the three methods corresponded with each other in 69,6%. Average agreement of the diagnostic methods was the highest between the two ELISA methods (74,3%), it was lower between the IDT and E1 (69,5%), and was the lowest between the IDT and E2 (65,1%). The average agreement of diagnostic methods by each allergen was the highest at hu (86,2%), fm (82,5%) and fe (75,5%) and then gr (68,3%), *A. siro* (65,1%), *D. farinae* (60,1%), we (59.9%) and *D. pteronyssinus* (59,8). The positive results of tests examined by pairs agreed in the lowest percent (under 10%) at fe, hu and fm at all three test matching, but the agreement of negative results was the highest (60-96%) for these allergens. The positive results at *D. farinae* agreed in high percentage; this was the highest between the E1 and E2 assays (69,7%).

In *Chapter IV* the ELISA test was in develoment, in the meantime by increasing sensitivity, the evaluations were made in two groups, first (m_1, before improvement) and second (m_2, after improvement). Results are indicated as follows: m_1/m_2. There were 50/150 atopic, 12/0 food allergic and non-atopic, and 7/12 clinically normal dogs. The most common positive test reactions were given by the next allergens during both tests: *D. farinae*, *A. siro*, *T. putrescentiae*, ragweed and mugwort. The positive correlations were the highest in *D. farinae* (46%/42%), *A. siro* (28%/37%) and *T. putrescentiae* (6%/43%); the overall total correlation was 76%/79%. The overall accuracy of ELISA was 69.6%/89% and of IDT 84%/75%. The ELISA had an overall sensitivity of 60%/80% and an overall specificity of 94.5%/100%. The IDT had an overall sensitivity of 80%/73% and an overall specificity of 94.5%/100%. The 58%/70% of atopic dogs had positive test reactions in both tests. The sensitivities of ELISA by each allergen were 100%/50% in grassmix,

100%/90% in *T. putrescentiae*, 93.2%/76% in *A. siro,* 74.4%/96% in mugwort, 40%/91% in *L. destructor* and 33.2%/63% in flea. Agreement of positive test results was the highest in *D. farinae* (72,7%/61%) and mugwort (71.4%/96%). Agreement of negative test results was the highest (100%/96%) in stinging nettle. 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. 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) in the first group. There was no correlation between them in the second group. The one aim of the development was to increase the sensitivity of imovet.bg allergy-serology test. The overall sensitivity (60%→80%) and sensitivities by allergens in 7 of 12 allergens (58%) managed to be increased. The positive correlation and agreement of positive test results increased in 67% and 50% of examined allergens. The evaluated improved ELISA test seemed reliable for the diagnosis of atopic dermatitis in practice comparing to the other commercially available tests and can be recommended for use in dogs when immunotherapy is a therapeutic option.

In *Chapter V* for the diagnosis of canine scabies, the sensitivity of the *Sarcoptes*-specific IgG ELISA test was 94.1% and the specificity 68.4%, calculated including the allergic dogs, and 85.7% with just the healthy dogs. Our results suggest that the IMOVET *Sarcoptes* specific IgG ELISA test is useful for ruling out a diagnosis of canine scabies. The interpretation of positive results may be difficult in atopic dogs sensitive to dust mites.

In *Chapter VI* seventy percent of the owners were satisfied with the effect of the hyposensibilisation. Symptoms were reduced by min. 50% in 11 cases (55%). In 70% of the cases concomitant therapies were needed. Half of the patients began to react to therapy between 3 and 6 months of the treatment. There were two peaks of the beginning of effect: on the 6th and the 16 th week 20-20% of the atopic dogs began to react, respectively. There was a large difference between the average time of existence of the symptoms before therapy between the patients who reacted well (9.2 months) and the patients who reacted poorly (19.5 months) to the hyposensibilisation. That is why the early diagnosis and treatment is recommended in the immunotherapy. The four patients who have been treated for 4 years didn't show any side effect during the time of the survey.

Conclusions

1. 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 total breed distribution in Budapest. Although Puli was not over-represented 5 of the 6 Pulis gave positive IDT reactions.
2. Males gave significant more ($p<0,01$) positive IDT reactions than females did.
3. 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.
4. 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.
5. Dalmatians have got significantly higher positive reactions to grasses than to house dust mites. German shepherd, Poodles, bulldogs, American staffordshire terriers, Labrador and Golden Retrievers, hungarian breeds were significantly higher positive to seasonal allergens.
6. 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.
7. INTEX Pharmazeutische Produkte AG developed two new ELISA methods (membran strip ELISA and microtiter plate assay) to measure the allergen-specific IgE in atopic dogs. INTEX had never had any test before which would have examined the canine serum, they only had serology-test for human sera. They developed their new tests on the basis only our 84 IDT results and canine sera. They have got nowadays their commercially available membran strip ELISA in Switzerland
8. After finishing the earlier project, Labor Laupeneck/imovet bg improved its allergy-serology test on the basis of our 231 IDT results and canine sera. This laboratory improved the sensitivity of the commercially available allergen-specific-IgE detecting ELISA test. ELISA had an overall sensitivity of 60% at the beginning and 80% in the second measurement.

9. The evaluated improved ELISA test seemed reliable for the diagnosis of atopic dermatitis in practice comparing to the other available tests and can be recommended for use in dogs when immunotherapy is a therapeutic option.
10. It was demonstrated that the IMOVET sarcoptes test is sensitive (94.1%) enough for the detection of antibodies to *Sarcoptes scabiei* in dogs. The specificity (68.4%) is not accurate enough to differentiate allergic skin diseases. The specificity (85.7%), calculated with just the negative control dogs, and sensitivity were 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.
11. Allergen-specific IgE-antibodies to *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae* mites and *Sarcoptes* mites were measured to evaluate possible immunologic cross reactions in atopic and scabietic patients. There is no similar study published examining the development of sensitivity to *A. siro* or to *T. putrescentiae* in canine scabies. Our study confirmed the existence of *in vivo* cross-reactivity between *Sarcoptes scabiei*, *D. farinae, A. siro*. and *T. putrescentiae*.
12. Based on our investigations the measurement of Sarcoptes-IgE is not an acceptable method for diagnosing either *Sarcoptes* mange infestation or Sarcoptes-allergy in dogs, according to our results.
13. Immunotherapy for canine atopic dermatitis was introduced by us in our clinic firstly in Hungary routinely. The 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. There were two “peaks” in the onset of improvement as 20% showed improvement at week 6 and another 20% improved at week 16.

Publications in canine atopic dermatitis

1. Refereed full-text research papers accepted for publication in scientific journals

1.1. In domestic journals, in Hungarian language

Király P., **Tarpataki N. (**2001) Tapasztalatok a kutyák atópiás dermatitisének vizsgálatával és kezelésével kapcsolatban *Magyar Állatorvosok Lapja*, 123. 15-22.

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

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

Tarpataki, N., Bagdi, N, Pápa, K., Papp, L. and Vörös, K. (2004): A specifikus immunterápia (hyposensibilisatio, desensibilsatio) és hazai alkalmazásának tapasztalatai atópiás bőrgyulladásban szenvedő kutyákban, 3. Specifikus immunterápia a gyakorlatban *Magyar Állatorvosok Lapja* **126**, 545–552.

1.2. In domestic journals, in foreign languages

Tarpataki, N., Pápa, K., Reiczigel, J., Vajdovich, P., Vörös, K. (2006): Prevalence and features of canine atopic dermatitis in Hungary. *Acta Vet. Hung*. **54** (3) (in press).

Tarpataki, N.: Recent developments in canine atopic dermatitis – a review. Submitted for publication to *Acta Vet. Hung.* (2006).

1.3. In foreign journal, in foreign languages

N. **Tarpataki**, B. Bigler, P. Vajdovich, K. Vörös: Evaluation of an enzyme-linked immunosorbent assay (ELISA) for serological diagnosis of canine scabies, Submitted in *Journal of Small Animal Practice* (2006)

N. **Tarpataki**, B. Bigler, P. Vajdovich, K. Vörös: Evaluation of an enzyme-linked immunosorbent assay (ELISA) 162 dogs. Planned for publication to *Journal of Small Animal Practice* (2006)

2. Refereed congress abstracts published in foreign languages in scientific journals with impact factor

N. **Tarpataki**, P. Csébi, K. Pápa, P. Vajdovich, K. Vörös The role of cytological and histopathological examination in small animal dermatology (Fifth World Congress of Veterinary Dermatology, Vienna, Austria, 2004. 08. 25-28.) (2004 ESVD and ACVD, *Veterinary Dermatology*, (15) (Suppl. 1) 41-69.

3. (Chapters of) Books, university textbooks and other reports published in edited non-periodicals

4. Congress papers published in proceeding books

4.1. Full text papers

Tarpataki N.: A nemi hormonok szerepe a kutya és a macska bőrbetegségeiben (Klinikus Állatorvosok Egyesülete, Kisállat Szekció (HSAVA) 12. országos konferenciája, Budapest, 2003. 05. 3-4. Összefoglaló: 15-18.)

Tarpataki N.: Kiegészítő vizsgálatok a bőrgyógyászatban. (Klinikus Állatorvosok Egyesülete, Kisállat Szekció (HSAVA) 14. országos konferenciája, Budapest, 2005. 04. 23-24. Összefoglaló: 36-40.)

4.2. Abstracts

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, Scientific Proceedings: 201)

5. Lectures and posters at congresses

Tarpataki N. , Magdus M.: Fürösztő-szerek alkalmazása a kisállat-praxisban (1998. 04. 18. I. Állategészségügyi Asszisztensi Konferencia, Budapest, ÁOTE) (Lecture)

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.: A nemi hormonok szerepe a kutya és a macska bőrbetegségeiben (Klinikus Állatorvosok Egyesülete, Kisállat Szekció (HSAVA) 12. országos konferenciája, Budapest, 2003. 05. 3-4.) (Lecture)

N. **Tarpataki**, P. Csébi, K. Pápa, P. Vajdovich, K. Vörös The role of cytological and histopathological examination in small animal dermatology (Poster: Fifth World Congress of Veterinary Dermatology, Vienna, Austria, 2004. 08. 25-28.)

Tarpataki N.: Kiegészítő vizsgálatok a bőrgyógyászatban. (Klinikus Állatorvosok Egyesülete, Kisállat Szekció (HSAVA) 14. országos konferenciája, Budapest, 2005. 04. 23-24.) (Lecture)

Tarpataki N.: Mit kell tudni a kisállatok parazitás bőrelváltozásairól ? (2006. 04. 22. Állatorvosi Szakasszisztensi Konferencia, SZIE, ÁOTK, Budapest)

6. Invited presentations (requested by foreign sister faculties, or organizing commities of national or international, non-congress like scientific meetings)

Tarpataki N.: A belgyógyászati tanszék kisállat-kórházának betegforgalma, különös tekintettel a bőrgyógyászati betegekre. I. Kisállat Bőrgyógyászati Szimpózium, 1997. 04. 10. ÁOTE, Budapest.

Magdus M. , **Tarpataki N**.,: Az allergiás bőrbetegségek differenciál-diagnosztikája. II. Kisállat Bőrgyógyászati Szimpózium, 2000.02.26., Hotel Agro, Budapest.

Tarpataki N.: Kutyák és macskák fiatalkori bőrbetegségei Pest megyei állatorvosi kamarai továbbképzés, 2001. 01. 25-26, Gödöllő.

Tarpataki N.: A magyar vizsla allergiás bőrbetegségei, különös tekintettel az atópiás dermatitisre. Magyar Vizslás Világtalálkozó, 2004.09.10-18., Kecskemét-Hortobágy.

Tarpataki N.: Allergiás bőrbetegségek diagnosztikája. VetMedLabor Szakmai Konferencia. 2004. 11. 24. Hotel Agro, Budapest.

Tarpataki N.: A pyoderma bőrtünetei és gyógykezelése kutyákban. Virbac Kisállat Szakmai Konferencia, 2004. 09. 25. Magyar Tudományos Akadémia Székházának Nagyterme, Budapest.

Tarpataki N. A pyoderma formái és gyógykezelése Magyar Állatorvosi Kamara Baranya Megyei Szervezete: X. Őszi Állatorvos Napok, 2004. 10.01-02. Harkány.

Tarpataki N.: Az eleségallergia differenciál-diagnosztikája. IAMS Kisállat Szakmai Konferencia. 2005. 03. 12. Rubin Hotel, Budapest.

Tarpataki N.: Allergodermatitisek gyógykezelése és megelőzése. Virbac Magyarország Kisállat Szakmai Konferencia. 2005. 04. 9. Magyar Tudományos Akadémia, Budapest.

Tarpataki N.: Allergodermatitisek gyógykezelése és megelőzése. Magyar Állatorvosi Kamara Baranya megyei Szervezete. 2005. 07. 7. Baranya megyei Állategészségügyi és Élelmiszer-ellenőrző Állomás, Pécs.

7. Further full-text professional papers published in non-scientific journal

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.)

Tarpataki N. A pyoderma bőrtünetei és gyógykezelése kutyákban (*Kisállatpraxis*, Különszám a Kisállat Szakmai Konferenciáról, 2-9. 2004)

Tarpataki N.: Kutyák atópiás dermatitisének gyógykezelésének lehetőségei. (*Kisállatpraxis*, 5. (1.) 6-13., 2004/1.)

8. Academian Reports

Tarpataki N. , Magdus M., Németh E.: Az atópiás dermatitis hazai előfordulása (Akadémiai Beszámolók, Budapest, ÁOTE, 1999. 01. 29.)

Tarpataki N. , Bagdi N. , Magdus M., Papp L., Vörös K., M. Dörffler, G. Emődi: Előzetes eredmények a kutyák intradermális allergiás bőrteszt jének 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 allergénspecifikus IgE típusú ellenanyagszintjének összehasonlítása (Akadémiai Beszámolók, Budapest, SZIE, ÁOTK, 2001. 01. 25.)

Tarpataki N., B. Beat, Oroszy A.: A rühösség diagnosztizálásának lehetőségei kutyákban, a diagnosztikai módszerek értékelése és összehasonlítása 29 klinikai beteg statisztikája alapján (Akadémiai Beszámolók, Budapest, SZIE, ÁOTK, 2002. 01. 24.)

Tarpataki N., Pápa K., Reiczigel J., Vörös K.: Kutyák atópiás dermatitisének jellemzői Magyarországon (Akadémiai Beszámolók, Budapest, SZIE, ÁOTK, 2006. 01. 26.)

9. Supervisor of diploma and scientific student research work

Szabó Orsolya: Az atópiás dermatopathiára gyanús kutyák in vivo és in vitro vizsgálata

Csébi Péter: A citológiai és kórszövettani vizsgálatok szerepe a kisállat-bőrgyógyászatban

Schubert, Christopher: The possibility of diagnosing the Sarcoptes-manginess in dogs, the valuation and comparison of the diagnostic methods on the basis of statistics of 29 clinical patients

Oroszy Andrea: A kutyák rühösségének diagnosztikai lehetőségei

Fábián Annamária: Az allergia-szerológia alapján végzett specifikus immunterápia hatékonysága atópiás dermatitisben szenvedő kutyákban

Nagy Szabina: A CD4/CD8 pozitív T-helper sejtek arányának megváltozása atópiás dermatitisben szenvedő kutyák perifériás vérében (TDK)

Dr. Horváth Oleszja: Az eleségallergia hazai előfordulásának jellemzői kutyákban

9. Awards

Tarpataki N., Bagdi Nóra, Pápa Kinga, Papp László, Vörös Károly *Márkus György Alapítvány díja: 2004., 2. díj:* A specifikus immunterápia és hazai alkalmazásának tapasztalatai atópiás bőrgyulladásban szenvedő kutyákban: A specifikus immunterápia a gyakorlatban