ABSTRACT

Key words: *atopy, dermatitis, diagnostic, clinical signs, treatment, glucocorticoids*

The doctoral thesis: ***Research regarding the diagnostics and treatment in canine atopic dermatitis*** fits in the general context of researches regarding the dermatological pathology in dogs.

The thesis´s purpose is to illustrate the clinical diagnostics and symptomatic treatment aspects, laboratory findings and bacterial complications that may occur.

The doctoral thesis summarizes a number of 232 pages being structured in 15 chapters, in which are included a number of 27 tables, 215 figures and color photographs and 183 references. First part of the paper work, concerning the current state of knowledge of the issues covered, includes introduction and the first 8 chapters, and the second part, represents the results of personal research, presented in 7 chapters, final conclusions, recommendations and references.

Atopic dermatitis is an allergic, inflammatory disease that follows a hipersensitivity reaction type I towards common airborne allergens. It can (extrinsic) or not (intrinsic) be accompanied by increased serum concentrations of specific IgE. The triggering factor is accomplished by exceeding the individual threshold value after the summation of multiple allergens. Besides the airborne ones, food, bacterial, fungal and parasitic allergens may contribute.

Atopy in dogs may manifest itself through skin lesions, respiratory signs but also external otitis, conjunctivitis, cheilitis, recurring pyoderma, yeast infections, digestive signs (flatulence, vomiting and dihareea).

Some breeds are known to be genetically predisposed to atopic dermatitis (Boxer, Chihuahua, Gordon Setter, Yorkshire Terrier, Shar Pei, West Highland White Terrier, Scottish Terrier, Lhasa apso, Shih tzu, Dalmatian, Mops, Golden Retriever, Irish Setter, Labrador, Cocker, Schnauzer, Belgian Shepherd, German Shepherd, Shiba Inu) and these, along with their crossbreeds, are more likely to have the disease or more severe clinical presentations.

The age of onset may be somewhere in between 6 months and 7 years with a high prevalence for patients of 1-3 years. External otitis may be the sole manifestation at onset, which complicates diagnostics. During its first episodes, atopic dermatitis may be seasonal (chronic

aspect afterwards), in which case its debut and flares will correlate with the presence of the original triggering allergens or with new ones.

The main allergens that are responsible for the manifestations in atopic dermatitis are airbone, stimulating the immune sistem both by respiratory and transcutaneous route. House dust mites are one of the most important allergen sources: *Dermatophagoides farinae* and *D. pteronissimus*, as well as *Euroglyphus maynei* (Solcan şi col., 2003), followed by polens (weeds, grass, trees), human and cat skin crusts, fungal spores (*Alternaria spp*., *Cladosporium spp*. etc.), storage mites, insect feces, plant and mineral particles, cockroaches, chemicals (Constantin, 2005). Even specific IgE for *S. scabiei var. canis* have been identified in atopic dogs (Solcan şi col., 2003).

The clinical manifestations of atopic dermatits are pruritus, erythema, papules, self inflicted alopecia though intensive licking and scratching, pustules, excoriations, erosions, lichenification, hyperpigmention. The localization of the lesions is important in ways that it may help orient the clinician towards a diagnosis of atopic dermatitis, food or flea allergy. Usually, the firs affected areas are the axila, the flexural surfaces, the abdomen, the inguinal area, the pinnae, the head especially periorbitally, the paws with a tendency towards pododermatitis. For the differential we may consider the dorso-lombar area as targeted in flea allergy dermatitis and the perinea in food allergy or food-induced atopic dermatitis (FIAD).

The most important cells in the skin of an atopic dog are the Langerhans cells and the dermal dendritic cells (responsible for processing and presenting allergens), B lymphocytes (synthesize reagines), T lymphocytes (cytokines production and stimulation of antibody synthesis in B lymphocytes) and mast cells (pro-inflammatory mediators) (Hill, 2001).

Mast cells exert their functions synthesizing and releasing pro-inflammatory mediators (Hill, 2001). In dogs, the histamine plasmatic concentrations are the same or even lower in atopic patients compared to normal, but the skin concentrations are always higher. Also, in atopic dogs mast cells are more sensitive than normal to both immunologic and non-immunologic stimuli (Marsella, 2001).

Th2 lymphocites stimulate the development of mast cells and eosinofils and also IgE and IgA production. In skin lesions from atopic patients Th lymphocites type CD3/CD4 are found. Th lymphocites CD4 are increased in lesional skin from atopic dogs compared to non-lesional or normal skin (Marsella, 2001).

The genetic defects that occur in the epidermal barrier (mainly the ones regarding structural proteins and lipids) are a condition for assuring the penetration of large molecular weight allergens such as house dust mites or pollens (Schlotter, 2009).

Besides the role that T lymphocites play in triggering the inflammatory process in the skin, they also act on the epidermal barrier. IL-4, IL-13 and IFN γ all contribute in inhibiting filagrine, loricrine and involucrine metabolism. IL-4 reduces the regenerating rate of the skins barrier function following aggression. Citokines, produced by Th2 lymphocites, reduce antimicrobial peptide production in the skin, thus increasing the risk of infections and further damage.

In order to establish a diagnostic the clinician will gather information from the anamnesis, the clinical exam, laboratory investigations and therapeutic response. The type, location, extension and severity of lesions will be investigated (Jasmin, 2011). It is necessary to exclude from the diagnostic all diseases that resemble atopic dermatitis or that coexist with it. Depending on history and clinical presentation the clinician will rule out mange, pyoderma, flea and food allergy, hormone disorders (Jasmin, 2011).

Maintaining a normal microflora on the skin is important in view of the fact that it confers protection from infections with pathogens or commensals (Olivry, 2011; Scott, 2001). *S. pseudintermedius* is known to be the main colonizer and pathogen of dogs skin and mucosal (Bannoehr, 2009). It is the most frequently isolated pathogen from pyodermas and otitis in dogs. It also produces a large variety of virulence and resistance factors such as: enzymes (coagulases, thermonucleases, proteases etc.), toxins (cytotoxins, exfoliative toxins, enterotoxins) and surface proteins (Futagawa-Saito, 2004, 2009; Gomez, 2014). Also, some strains may produce biofilm (Futagawa-Saito, 2009).

The treatment in atopic dermatitis may be specific, desensibilization, or symptomat (systemic or local administered drugs). Glucocorticoids may be used both ways, also antihistamines, fatty acid dietary supplementation, vitamins, minerals and imunomodulators. All these may contribute in regulating the pro-inflammatory mediators sinthesys (prostaglandins, leucotriens), inhibiting lymphocyte activation and cytokine release, preventing the structural and functional damage of the epidermal lipid layer. Ciclosporin A, an antiinflammatory selective imunosuppresor, is an alternative to glucocorticoids in treating canine atopic dermatitis that proved its efficacy in more than 80% of patients (Marsella, 2001).

Personal results

Epidemiological aspects in canine atopic dermatitis

The research was done on 6998 dogs (34 breeds with 9 individuals or more and crossbreeds) with a variety of diseases, registered in the Medical Clinic of the Veterinary Medicine Faculty of Iaşi, between 2008 – 2015.

Out of all the dogs with skin problems (8.9% of the general population) the allergic skin diseases had the highest percentage (67.7%). Of all the allergic based dermatitis (atopy, flea and food allergy) the atopic one had the highest number of cases (59.9 %).

Most of the atopic patients were registered in 2010, a year with an exceedingly rainy weather, while 2011, 2012 and 2013 were exceedingly dry years (182).

Regarding the seasonal distribution, most cases were registered during summer, june-august and few during spring. These data match the vegetation, blooming and pollination calendars of the plants in our country (Hodişan, 2008).

Most of the patients were crossbreeds (26.6%) or Pekingese (11.6%). Some of the breeds susceptible to dermatitis were weakly represented in the general population: German Shepherd 9.7 %, Labrador 3.4 %, WHWT 1.5 %, Bulldog 1.4 %, Shar Pei 1.4 %.

The occurrence of skin problems among the dogs belonging to a certain breed was sometimes surprising: WHWT 40.5 %, Pittbull 36.8 %, Bullterrier 33.3 %, Shar Pei 30.2 %.

Out of all skin diseases from inside a breed population, atopic dermatitis was highest represented in Collies, Setters, Schnauzers, Fox Terriers, WHWT, Shar Peis, Tekels, Pittbulls.

From the general population 3.6% of all cases were atopic dermatitis, spread among the breeds as follows: crossbreeds 18.8 %, WHWT 11.8 %, German Shepherd 9.4 %, Pekingese 8.6 %, Labrador 6.6 %.

Age distribution of the cases illustrates that, usually, atopic dermatitis has its debut between 1 and 3 years (42.9%), but can occur from 6 months to 8 years.

Clinical diagnosis in canine atopic dermatitis

The clinical study was done for 140 pacients: 62,2% females and 37.8% males. Regarding the environment, 10% of the dogs were from the rural area whilst the majority of 90% were from the city. Almost 60% were strictly indoor dogs, going out just for 2-3 short walks. The rest of 40.7% of dogs were staying outdoors or both.

Besides the skin lesions, non-dermatological clinical signs were noticed in the atopic patients considered for this study: 25% of them had flatulence, 5.7% occasional vomiting and 30.7% had enlarged lymphnodes. In 18.3% of the female patients were noticed that the

cutaneous signs were concomitant or shortly followed heat period (6 cases), giving birth (4 cases) or false lactation (6 cases).

All dogs received mixt commercial food, usually based on chiken meat or chiken and fish. Almost 15% of patients were already receiving a hypoalergenic diet when first presented for consult. Owners decided a dietary change for 20% of the patients, after the onset of the dermatitis in hope that it may improve clinical signs. Home cooked food was gived to 55.7% of dogs.

Treatment for external parasites was given regularly only to 35.8% of all patients, for the rest of them it was either irregular (34.2%) or lacking (30%).

Atopic dermatitis first started with pruritus in 60% of cases and with external otitis in 40%. Other symptoms that were observed were cheilitis in 10.7% of dogs, conjunctivitis in 24.2%, sneezing in 2.8% and asthma in 1.4%.

Most dogs first presented with a mild form of atopy (7% had only erythema and papules), only 23.5% of them had secondary lesions like hyperpigmentation, 20% lichenification and 21.4% redish tint of the fur due to intensive licking. Lesions due to scratching were mostly excoriations (43.5 %) but erosions (4.2 %) and plagues (5%) were also found. Seborrhoea was seen in 30% of patients.

Regarding the location of the lesions, the abdomen was frequently affected in 79% of dogs, presenting secondary lesions in 20.7% of cases. The ventral neck and chest areas were targeted in 34.2% patients and 27.8% respectively.

The inner thies presented both primary and secondary lesions in 70% of patients, sometimes extending downwards towards the paws in 12.8% of dogs.

The inguinal region was affected in 65% of dogs, with lesions sometimes involving the genitals: hyperpigmentation, lichenification, fistulas, bacterial or yeast infections favoured by the moist environment of the area and the microlesions that appear between skin folds.

Front paws presented lesions in 37.8% of dogs, whilst back paws only in 35.7%. Bacterial and yeast pododermatitis was also observed.

In this study, 22% of dogs presented with periorbital alopecia and erythema, and sometimes hyperpigmantation. Of all patients, 24.2% had lesions on their face other than periorbital, the pinna or lips.

În German Shepherds and their crossbreeds we observed a higher tendency of lesions towards affecting the dorso-lombar area and their constant presence on the ventral neck area.

Periorbital alopecia and erythema along with papular eruption on the pinna were especially seen in Labradors. External otitis was found in all breeds, but with more severe presentations in WHWT where the ear canal was frequently blocked with secretions and inflamed tissue.

In WHWT we observed that most of the patients had all the ventral areas affected by extended secondary lesions and either pyoderma or yeast infections (*Pseudomonas spp.*, *Malassezia spp.*).

Laboratory findings in canine atopic dermatitis

Out of 15 dogs examined, 6 showed decreased Ca levels and in 4 these were correlated with low values in the total protein level. The CBC revealed a slight anemia, macrocytosis, active monocytes, low hemoglobin or hemoconcentration.

In 7 out of 15 cases, there was an obvious response to the inflammatory process inside the dermis. Lymphopenia and eosinophilia were the main findings associated with neutrophilia and the left deviation of the Arneth index indicating a strong corticoid reaction with systemic stress and the start of an infection (increased WBC), most likely a superficial pyoderma.

Bacterial complications in canine atopic dermatitis

We isolated 60 bacterial strains (50 *S. pseudintermedius*: 20 methicillin-susceptible from România - MSSP-RO, 20 methicillin-susceptible from the UK - MSSP-UK, 10 methicillin-resistant from the UK - MRSP-UK; 10 coagulase-negative staphylococci from the UK - CoNS-UK) from dogs with atopic dermatitis from România and the UK. Sensitivity tests were performed for 12 antimicrobial substances and the presence of resistance (*mecA, mup, fusB, fusC, fusD*), eflux pumps *(smr, qac A/B*), virulence (*lukS, lukF, siet, sec$_{canine}$, expA*) and biofilm producing genes (*icaA, icaD, bap*) was investigated.

MSSP-RO expressed resistance towards a higher number of antimicrobials than MSSP-UK (amongst which gentamicin and chloramfenicol) and sensitivity for ampicillin, their resistance pattern being more similar to MRSP-UK. For MRSP-UK we observed high resistance for ampicillin – 70%, fusidic acid – 45% and tetraciclin – 30% and a tendency towards sensibility for the other antimicrobial substances tested.

Leukocidins, *lukS şi lukF* were the most common virulence genes in all isolates: 100% in MRSP-UK, 95% in MSSP-RO and MSSP-UK. The toxins encoded by these genes are likely to have an important role in the pathogenesis of pyodermas in dogs. We only identified 4 virulence genes in MRSP-UK (*lukS, lukF, siet, expA*). The *siet* gene encodes an exfoliative toxin with

clinical effects in pyodermas (Bannoehr, 2012). This gene has been previously identified as a contributing factor in impetigo in dogs (Masayuki, 2009).

The *sec*$_{canine}$ gene was found in 30 % of MSSP-RO and 15 % of MSSP-UK and the *expA* gene in 20 % of MSSP-RO and in 10 % of MSSP-UK.

Regarding the resistance genes, the isolates that harbour fusidic acid resistance genes (in 21% of isolates) also manifested phenotypic resistance. We identified one MSSP-RO with the *qacA/B* gene and one MRSP-UK with *qac A/B* and *smr*. In CoNS we found 60% of isolates harboring the *qac A/B* gene. We found the mupirocin resistance in 3 CoNS isolates but no sensibility tests were performed.

Symptomatic treatment in canine atopic dermatitis

From the initial studied group, only 104 dogs diagnosed with atopic dermatitis received treatment according to a personalized therapeutic scheme. Symptomatic treatment was based on orally administered glucocorticoids, antihistamines, essential fatty acids, vitamins, minerals, liver protectors, probiotics, medicinal shampoos, medicinal ear drops, external antiparasitics, antibiotics, antifungals and restriction or hypoallergenic diets.

All patients received prednison, oral pills of 5 mg each. Dosage varied from 0.5 mg/kg per day to 0.75 mg/kg per day, with a treatment period from 4 up to 14 days, depending on clinical presentation on consult and therapeutic response. After 1-2 weeks Prednison doses were cut to half, 0.3-0.25 mg/kg per day for another 2 weeks. For the patients that returned for consult and depending on the evolution of their clinical signs, the treatment was continued for another 1-4 weeks.

Glucocorticoid treatment targets mainly reducing the pruritus and local inflammation. In order to cut down the prednison dose faster than in monotherapy and still maintain the therapeutic response, we associated type I antihistamines in 102 patients: 68.6% clemastine, 17.6% cetirizin and 13.7% desloratadine. All were administered as oral pills but cetirizin also as a syrup, which allowed accurate dosage in small dogs.

All patients had a positive response to treatment, reduced pruritus by 75% after 1-2 days from the first administration. Regarding antihistamines, the best results were seen when clemastine was used, followed by cetirizin. In some patients it was even possible to eliminate glucocorticoids completely after 3-4 weeks and maintaining a normal clinical status with only antihistamines.

Aditionally we used medicinal shampoos, mostly with 4% chlorhedixine, in 56.7 % patients. These baths significantly reduced pruritus, bacterial, yeast and allergen load from the skin and coat.

To rule out external parasites, in 37.5% patients we recomended that an efficient deparasitasion would be done using: for 10.2% imidacloprid+permetrin, 51.2% imidacloprid+moxidectin, 23% selamectin, 10,2% afoxolan and 5% fipronil.

Treatment was completed with essential fatty acid dietary supplementation, vitamin and mineral complexes and, in 13.4% patients, also oral drops of vitamin D.