

ABSTRACT

The doctoral thesis entitled „***Researches regarding autoimmune diseases dogs and cats***” has as a start point, first of all, the restrained area of researches regarding these diseases in România, the practical aims being to establish the distribution of the autoimmune diseases in cats and dogs on Moldavia land, in function of breeds, ages and sex, evaluation of haematological, immunological and immunohistochemical parametres to establish some correlations regarding their changes once the autoantibodies appear, to test the sensitivity and specificity of some immunological diagnostic tests which can ascertain a diagnostic of autoimmune disease, as well as a modest trying of finding some scientific and practical correlations between the feline infectious peritonitis and other autoimmune diseases in cats.

The thesis contained 415 pages and is structured, in accordance with legal stipulation, in two main parts: first part, entitled “***Actual stage of knowledge***” include 117 pages, 8 tables, 6 photos and the second part “***Personal contribution***” covers 223 pages, 332 tables, 12 charts and 119 photos which illustrate the results and simplify the understanding of text.

First part covers five chapters in which are briefly and concisely presented informations from scientific literature focused on the topic of the thesis data that were used to interpret and compare the results obtained in second part of the thesis.

First chapter intitled “***Immunity and immune system***” covers 4 subchapters in which the component structures of the immune system (primary and secondary lymphoid organs, cells with role in immunity, molecules of the immune system) and their way of interaction in order to assure the immunological homeostasy of organism are presented.

In the 3 subchapters of the 2nd chapter intitled “***Tolerance and autoimmunity***” are presented datas from the speciality literature related with the capacity of the immune system to recognize and tolerate the self, various factors which produce the rupture of

these state and the installing of autoimmunity state, as well as the appearance and the way of action of the autoantibodies.

Chapter 3 entitled “**Generalities regarding autoimmune diseases**” comprises 4 subchapters in which are presented shortly a classification of the autoimmune diseases conform to datas existing in speciality literature, factors which favourize the installing of an autoimmune disease, as well as general mechanisms of tolerance rupture. A special importance is accorded to cellular and molecular mechanisms of autoimmune diseases progression, systemic ones and organospecific ones.

Chapter 4 entitled “**Autoimmune diseases**” has 9 subchapters in which are presented datas regarding systemic autoimmune diseases (systemic lupus erythematosus, rheumatoid polyarthritis, amyloidosis, dermatomyositis, Sjögren syndrom), as well as organospecific diseases of the endocrine glands (Hashimoto thyroiditis, insulin dependent diabetes, hypoadrenocorticism – Addison disease), of the central nervous system (myasthenia gravis, pug dogs encephalitis (PDE), allergic encephalitis, coonhound paralysis, steroid treatable meningitis), of blood (autoimmune haemolytic anemia– AHA, cold haemagglutinins disease, autoimmune thrombocytopenia), of blood vessels – vasculitis mediated by immune complexes, bullous skin autoimmune diseases (pemphigus, pemphigoid bullous, alopecia areata, linear dermatosis with IgA, psoriasis), eye autoimmune diseases (Keratoconjunctivitis sicca – CIKS, Vogt-Koyanagi-Harada syndrom – VKH, optical neuritis, autoimmune uveitis) and some other autoimmune diseases like polymyositis and myositis. Another subchapter is dedicated to the therapy of the autoimmune diseases.

Last chapter of the first part is dedicated to feline infectious peritonitis, with datas referring to etiopathogeny, clinical signs, prevention and control of this diseases.

The second part „**Personal contributions**” comprise seven chapters, in which the results of investigation are presented and discussed.

In chapter 6 entitled “**Methods used in autoimmune diseases diagnostic**”, during the 11 subchapters the materials and methods used in researches to evaluate humoral immunity (detection of antinuclear antibodies – ANA, rheumatoid factor– FR, C reactive protein – PCR, antistreptolysin O antibodies – ASO, evaluation of immunoglobulin G titre, Coombs test (antiglobulinic), dosage of the complement through haemolysis 50%

method, detection of cryoglobulins) and tests to evaluate the cellular immunity (evidentiation of lupic cells, haemoleucograms and leucocitar formulas), as well as histopathological exam are presented.

In chapter 7 entitled “*Clinical cases presentation*” the 28 cases diagnosticated with autoimmune diseases during the study are described, classification being made on syndroms and associations of clinical symptoms: systemic lupus erythematosus (dermatitis and immune nephritis, dermatitis with polyarthritis, polyarthritis with immune nephritis, dermatitis), discoid lupus erythematosus, rheumatoid polyarthritis, rheumatismale fever.

In chapter 8 results obtained in the four years of research for the autoimmune diseases in dogs are presented. From the entire spectrum on maladies we diagnosticated only four (systemic and discoid lupus erythematosus, rheumatoid polyarthritis and rheumatismal fever).

Researches were effectuated during 2003-2007 on a number of 100 animals, 60 of them being suspected by autoimmune diseases (45 dogs and 15 cats), and the other 40 constituting the control plot of healty animals (20) and plot of animals with some affections, others then autoimmune.

After the investigations were made, it was observed that in dogs, from the 45 cases, only 20 were diagnosticated with systemic lupus erythematosus: 3 cases of chronic dermatitis, associated with immune chronic nephritis, 8 cases with polyarthritis, maniffestations of slow medullary compresion and immune chronic nephritis, 2 cases with dermatitis and polyarthritis, 7 cases with dermatitis resistent to antibiotic treatment, with all the immunological test positives.

Discoid lupus erythematosus was diagnosticated in 2 dogs, 3 cases of rheumatoid polyarthritis and rheumatismal fever each.

Skin lesions were observed in 60% cases, nonerosive polyarthritis in 50% cases, and kidney diseases in 11% cases.

The antinuclear antibodies titre detected in all case s evaluated through latex agglutination test registred different values between 1:32 and 1:1024. In 6 cases (5 with Systemic lupus erythematosus and 1 with discoid lupus erythematosus), representing

21,42%, determination was pure qualitative, and in 3 cases (all of them diagnosticated with rheumatismal fever), representing 10,71%, results were negatives.

Indirect immunofluorescence reaction was used only in 5 cases with agglutination reaction positive, with titres between 1:512 and 1:1024. After serums dilution we obtained positive reaction only in one case.

We can conclude that in all cases of systemic lupus erythematosus titre was correlated with the gravity of clinical symptomatology, and big titres of 1:512 and 1:1024 were correlated with the severity of the clinical signs, and the presence of lupic cells.

In order to detect the presence of rheumatoid factor, reaction was positive through both tests (latex agglutination and Rose Waaler) in from the 28 animals, only by Rose Waaler test in 5 animals and negativ in 14, the titre being between 16 and 136 U.I./ml. Using the latex agglutination test the rheumatoid factor was detected in the synovial fluid in a titre of 1:32.

C reactive protein registred values between 180 and 600 mg/dl, being detected in 21 from the 28 cases, at the other 7 being negative.

Antistreptolysine O antibodies were detected only in 3 cases of rheumatismale fever, in the other 25 cases being negative.

IgG titres were detected in 16 from the 28 cases. The alues were decreased in 3 cases with immune and dermatitis or polyarthritis, all of them presenting cryoglobulins. Al the other 11 cases IgG titre was increased, with values between 17,9 and 30,5 mg/ml.

Autoimmune haemolytic anemia was observed in a german shepard female, with the Coombs test positive meanwhile thrombocytopenia wsa observed in 2 cases values being og 124 and $62 \times 10^3/\mu\text{l}$.

Reffering to the cellular immunity the lupic cells were observed in 7 cases: 1 case of dermatitis and nepritis, 3 cases with polyarthritis and nepritis, 3 cases of dermatitis.

The histological skin examination in dogs with dermatitis diagnosticated with systemic lupus erytematosus were observed the following aspects: cells from basal and mucous Malpighi sheet of epidermis have prismatic aspect and thre are loaded with melanine realizing the pigmentary incontinence, the basale membranes of epidermis, blood capilary, sudoripary glands and epithelial sheets of the hair foliculs are thicker, with paracheratozis and achantosis in the superficial sheet, dermic sclerosis, degenerated

hair follicles or follicles in which epithelial sheets are formed by prismatic cells, loaded with melanine, inter- and perifollicular lymphocyte infiltration.

At the radiologic exam the thickening of the intervertebral discs in lombosacral or dorsal region and the slight ventral deviation of two of them was observed.

The manifestation of immune glomerulonephritis were observed in 11 from the 20 cases diagnosed with SLE. 3 from them presented dermatitis and 8 of them, polyarthritis. At the biochemical exam of the urine moderate proteinuria (0,8 – 1,8 g/l) was observed, the other tested parameters being unmodified.

In chapter 9 entitled “*Autoimmune diseases in cats*” are presented the 3 clinical cases and the results obtained after the evaluation of immunological and haematological parameters.

From the 15 cats suspected by autoimmune diseases, the diagnosis of systemic lupus erythematosus was established in 3 cats.

The perturbation of the humoral immunity was translated by the detection of the antinuclear antibodies using the latex agglutination test in all 3 cases, the titres obtained being of 1:128 in 2 cases and 1:64 in one case.

The reaction to detect C reactive protein was positive, with titres of 300 and 240 mg/ml in two cases. Antistreptolysin O antibodies weren't detected, but the rheumatoid factor was detected only in one cat using the latex agglutination test, with a titre of 40 UI/ml.

In chapter 10 entitled “*Epidemiology of the autoimmune diseases*” are presented data referring to the distribution of the diseases on breeds, ages and sexes.

The 28 animals diagnosed with autoimmune diseases made part the following categories: 9 from German shepherd (5 females and 2 males with SLE, a female with rheumatic fever and a male with rheumatoid polyarthritis), 5 from the common breed (3 female with SLE and a female and a male with rheumatoid arthritis), 3 from Pekinese breed (2 females and a male with SLE), 3 from Rotweiler breed (a female and 2 males with SLE), 1 each from the Collie breed (male with SLE), Cocker (female with SLE), West Highland Terrier (a male with SLE), Boxer (a female with SLE), 1 male each from Akita Inu and Alaskan Malamute breed with discoid lupus erythematosus and 1 Bishon

maltez male and a Chow-Chow female with rheumatoid fever, different ages (from 1 to 11 years).

Chapter 11 entitled „*Results regarding feline infectious peritonitis*” presents the materials and methods used in the study of the disease and the obtained results.

The study for the feline infectious peritonitis was effectuated on 30 blood and 8 feces samples.

Using the RT-PCR technics, coronaviral RNA was detected only in the feces from the 3 cats (FCoV-57, FCoV-60 and FCoV-61), revealing the fact that the animals were exposed to the feline coronavirus, indirect immunofluorescence being positive only in one of them (titre of 1:625).

Except the samples from the cats with the positive virusological tests, there were tested 20 serum samples only for the presence of the specific antibodies, the titre obtained being of 1:625 in 5 cases.

All the samples came from cats who were in contact with dogs and other cats.

There were tested also 2 ascitic fluid samples from cats suspected by FIP, the coronaviral RNA being found only in one case.

Besides the virusological test, there were performed other immunological and haematological tests which revealed the presence of antinuclear antibodies in two cats, the neutrophilia and lymphopenia.

Laboratory exams were related with the clinical examination results, in case of the autoimmune diseases diagnosticated in dogs and cats, as well as in the case of feline infectious peritonitis.

Is very important to remember is the fact that any suspicion comes from a symptomatology. What is coming after, to confirm or to infirm the suspicion depends of the test sensitivity and specificity, specially serological and virusological. To confirm a diagnosis of viral infectious disease there must be realized virus isolation and identification, using technics of molecular biology (PCR, RT-PCR) or specific antibodies recognition