ABSTRACT

Keywords: diagnosis, nervous system, kidney diseases, small animals

The purpose of this thesis is to present the most important methods of diagnosing kidney disease in small animals, but mainly to highlight ways to evaluate and identify neurological phenomena that sometimes accompanies these illnesses.

The first part of the thesis consists of 2 chapters dealing with the current stage of knowledge on the diagnosis and etiopathogenesis of uremic encephalopathy and the main nephropathies in small animals.

Chapter 1 presents the peculiarities of neurological manifestations in uremic encephalopathy, the common aspects of human medicine as well as modern diagnostic tools.

Chapter 2 of the thesis contains notions of etiopathogenesis, clinical manifestations and ways to diagnose kidney diseases encountered in small animals. For the classification of nephropathies, the criterion of localization of the pathologic process was used as follows: in glomerular, tubulo-interstitial, then vascular, hereditary, mechanical and neoplastic.

Part 2 includes personal research, structured in 4 chapters, including the material and working methods, the results obtained, their interpretation and the general conclusions.

The researches took place between October 2013 and April 2017, were 3730 patients were consulted at the Medical Clinic of the Faculty of Veterinary Medicine in Iasi, from which 2517 dogs and 1213 cats.

Of the total patients, 163 were diagnosed with kidney diseases, with or without obvious clinical signs, representing a proportion of 4.36% of all the diseases diagnosed in the mentioned period. Patients underwent general clinical examination using the clinical examination sheet as well as complementary diagnostic examinations: ultrasound and biochemical examinations of blood and urine. The clinical examination was conducted following the order of general semiological methods. During consultation, the patient identification data of the species, race, age, and further information from the anamnesis, following the discussions with the owners, are noted in the clinical examination card.

The presumptive diagnosis is based on anamnestic data and later paraclinical investigations. The diagnosis of acute or chronic renal failure has been established after the time of the onset of clinical manifestations has been identified observed by the owner prior to consultation (about three months in the case of chronic or sudden renal insufficiency in case of acute renal failure), depending on the age of the patient, but also on the ultrasound aspects of the kidneys.

The blood sampling was performed predominantly from the cephalic and jugular vein, 3 to 5 ml of blood was drawn and distributed into cloth activator tubes and EDTA tubes.

For both cats and dogs, renal impairment was established according to the staging system set by IRIS in 2013.

Statistical analysis of the data was performed using the IBM SPSS 21 software. The variables considered for the analysis refer to the degree and type of renal failure, creatinine and urea value and general clinical signs such as: vomiting, anemia, the presence or absence of urinary tract infections, heart disease, immunizations, as well as temperature, heart rate, respiratory rate, and type of food. Most clinical signs reported in kidney failure are the consequences of uraemia, the uremia being the clinical stage to which all kidney problems convert at the end. The first clinical signs considered in the statistical analysis were the urinary ones, of which the presence of urinary tract inflammation - cystitis, which was reported in 25% of patients. Polyuria / polydipsia occurred in 87.5% of patients, and refers to the formation and elimination of excessive amounts of urine that may exceed 50 ml/kg/day and polydipsia at increased water and fluid consumption that exceeds 100 ml/kg/Day. The most commonly mentioned digestive signs were changes in appetite and transit, and vomiting. Selective appetite was reported in 45% of patients, 55% lacked appetite. Vomiting was reported in 50% of patients, being caused by the effect of a series of unidentified toxins on the center of vomiting and uremic gastritis.

Pica was manifested in 20% of patients by occasional consumption in small amounts of slurry litter followed by vomiting most of the times. Pica is referred to in the literature as being a clinical sign of anemia especially that associated with iron deficiency. Anemia was reported in 55.6% of patients.

In 4^{th} chapter there were presented the main diagnostic tools used in kidney failure patients and their results.

The statistical analysis of the data was performed using the IBM SPSS 21 software, using the Mann Withney and Ttest paired samples with a significance threshold set at $p<0.05$. The variables considered were: species, sex, degree of renal insufficiency, type ofrenal insufficiency, creatinine, urea, potassium, sodium, phosphorus, magnesium, total calcium, protein, hepatic enzymes (AST, PA, ALT, GGT), cholesterol, iron, erythrocytes, hematocrit, hemoglobin, MCV, MCHC, leukocytes and the proportion of lymphocytes, granulocytes, basophils, monocytes, eosinophils and reticulocytes.

Hyperphosphatemia is the most common mineral imbalance in patients with chronic renal failure. Changes in potassium metabolism in this group of patients have been associated with hypokalaemia. Hypokalaemia is an electrolyte deficiency mainly in CKD cats. In dogs with this condition, hypokalaemia is not commonly observed. There are studies that claim that long-term hypokalaemia may be the cause of the progression of renal failure in cats, and not just a consequence of it. Increased serum total calcium concentrations have been reported in patients with CKD occurring primarily because of increased calcium complexation with organic and inorganic anions that may have higher concentrations in CKD such as citrate, phosphate or sulphate.

From the determined liver enzymes, the AST was above the upper limit.

In renal failure, due to chronic blood loss and erythropoietin deficiency, anemia often occurs with disease progression. Anemia in this pathology has characteristic traits, being

normochromic, normocytic, and hypoproliferative. Systemic stress in this pathology is characterized by the presence of neutrophilia and eosinopenia, which was also observed by the negative correlation (r^2=-0.773, $p<0.05$) between the value of neutrophils and eosinophils.

The imaging investigations of this disease were carried out on of 24 cats and 28 dogs. The most common lesional aspects, consistent with ultrasound images, were cortical sclerosis, medullary sclerosis, inflammation of the pelvis and hydronephrosis.

Chapter 5 discusses the ways to diagnose neurological disorders that occur in renal failure. 10 patients, 5 dogs with chronic kidney disease aged 12-18 years and 5 dogs with acute renal failure caused by ethylene glycol intoxication aged 4 to 9 years were enrolled in the bioelectric brain activity study. The group of cats consisted of 6 patients, 3 diagnosed with AKF and 3 with CKD, aged between 2 and 17 years.

All patients were sedated before recording EEG using 0.03 mg/kg of medetomidine hydrochloride (Domitor, Pfizer) administered intramuscularly to reduce the frequency or appearance of artefacts due to muscle contractions. The acquisition of EEG traces was performed with the Neurofax (Nihon Kohden) electroencephalograph for a period of 30 minutes. Needle-type electrodes were placed subcutaneously after pre-trimming and degreasing of the skin surface with alcohol, using the Redding model. The parameters used for each electroencephalographic recording are: sensitivity: 70 μV, time constant: 0.3 seconds, trace filter of 70 Hz and electrode impedance <10Ω. All EEG records were visually analyzed; artifacts were determined by direct tracking of records, background activity being analyzed during registration to identify any detectable changes.

In both CKD and ethylene glycol poisoned dogs, the bioelectric activity of the brain was characterized by non-specific electroencephalographic abnormalities: diffuse delta waves and slow background activity. No interictal epileptic discharges (characteristic of idiopathic epilepsy) were observed.

EEG traces had a low voltage appearance and initially had a slow background activity of 5-7 cycles / second, over which diffuse delta and bilateral synchron delta was observed, being the expression of a cortical or subcortical neuronal hyperexcitability state. The background activity was characterized by high amplitude and low frequencies, the most likely changes in the use of medetomidine.

During the recordings it was possible to notice the occurrence of electric crises on all branches, which were characterized by a sequence of peaks with a frequency of 15-35 cycles/second, which gradually increased in amplitude, reaching values up to 250-500 μV.

In feline patients, non-specific electroencephalographic abnormalities similar to those seen in dogs were identified: where beta and diffuse theta, background activity slowed. Also, no interictal discharges specific to idiopathic epilepsy have been identified. Other changes observed in all patients were sparing medications, fast, diffuse rhythms, commonly found in patients who received benzodiazepines or other psychotropic medications.

Quantitative analysis of EEG tracts in cats included 5 patients, 2 with IRC and 3 with AKF. For each patient, 30 EEG epochs were selected without artifacts and

paroxysmal events of 2 seconds each. The number of alpha waves (8.0-13.0Hz), beta (13.0-30.0 Hz), delta (0.5-4.0 Hz) and theta (4.0-8.0 Hz) expressed as relative power (%) were calculated for each channel. After comparing the relative frequencies of the alpha, beta, theta, delta waves, no statistically significant differences were noted depending on the stage of evolution of the disease, or between the sedated patients and those whose EEG tracing was done without sedation. The overwhelming presence of beta and theta waves was observed, with the absence of delta waves in the two patient groups. Given that beta waves are predominante in wake state, and the theta waves in the early stages of sleep, we can say that the electroencephalographic traces studied in terms of quantitative analysis are within normal limits.

The study on the metabolism of cerebrospinal fluid in uremic encephalopathy was performed on 10 healthy dogs aged 2-5 years and weighing 15-20 kg dogs (control group) without a history of neurological disease and 7 dogs with end-stage renal failure and a dog with a ruptured urethra (CKD group), also diagnosed with IRC, aged 8 to 15 years and weighing between 4.5kg and 32kg. Patients in the CKD group have never been diagnosed with any neurological condition.

The collection of CSF samples was performed on patients on general anesthesia with medetomidine (Domitor, Pfizer) 0.03 mg/kg and ketamine (Kepra) 0.1 mg/kg administered intravenously, from the cerebellumedular cistern with a spinal needle with a diameter of 22.

Sample analysis was performed at a temperature of 26.5°C using a Bruker Avance III 400 MHz spectrometer operating at 400.13 MHz with a 5 mm inversion multi-nuclear detection probe equipped with gradients on the z-axis.

For healthy dogs, 18 metabolites were identified and 21 were quantified for the CKD group. Succinic acid, dimethylamine and tyrosine are missing from the control group; these metabolites have not been identified in healthy dogs. Notable differences were observed in the levels of creatinine, lactate, acetic acid, acetone, pyruvate, creatine and myo-inositol between the healthy group and the CKD.

Evaluation of autonomic nervous system disorders by heart rate variability aimed to evaluate simpato-parasympathetic (sympathetic) balance in dogs with chronic renal failure using EKG monitoring over a short period of time, 5 minutes being sufficient to obtain the parameters needed for the investigation. The study included 13 dogs (different ages and breeds) with chronic renal failure (CKD group) diagnosed according to the IRIS guidelines but without detectable cardiac conditions. To compare the results of the HRV analysis, we used the parameters obtained from 13 healthy dogs (without heart or kidney disease) of the same gender with similar age and weight.

To obtain the EKG recordings, the PolySpectrum - 8E/8V (Neurosoft) electrocardiograph was used, and the Kubios HRV Analysis Software (Kuopio University, Finland) was used to obtain the HRV parameters. Statistical analysis of the data was performed using the IBM SPSS 21 software, using the Mann Withney and Ttest paired samples with a significance threshold set at $p < 0.5$. The most commonly used methods for the analysis of heart rate variability are time domain analysis and frequency domain analysis.

We evaluated the time and frequency domain parameters to estimate global HRV (SDNN), vagal activity (pNN50 and HF) and sympathetic tone (LF and LF/HF). Our results showed a significant decrease in overall HRV (SDNN) ($p=0.017$) in the CKD group associated with a significant decrease in vagal tone (pNN50 and HF). Moreover, sympathetic activity is increased ($p<0.001$ and $p<0.05$) in the CKD group compared to the healthy group. Our study has shown that patients with CKD show increased sympathetic activity associated with decreased vagal activity, with overall decrease in cardiac variability. This autonomous imbalance is an indicator of a bad prognosis in dogs with heart disease.

Chapter 6 included the study of histopathological and electronomicroscopic aspects in kidney diseases performed on a number19 dogs ranging in age from 7 months to 11 years, and 9 cats aged 1.5-17, diagnosed with AKF or CKD, and uremic encephalopathy, performed on 8 dogs.

Renal lesions recorded with the highest frequency in dogs are: membrano-proliferative glomerulonephritis and glomerulosclerosis (24%), lymphohistiocytic nephritis (12%), tubulonephritis characterized by epithelial degeneration (14%) and the presence of casts (14%) of proteinuria. In cats the most common injuries to the patients studied were: glomerulonephritis (25%), tubulonephritis (18%), the presence of casts (14%) and crystals of oxalate or urate (14%).

In uremic encephalopathy, the most commonly encountered lesions were: congestion, cerebral edema, and tissue vacuoles.

The last chapter outlines the general conclusions of the studies presented.