SUMMARY

The doctoral thesis entitled **„Researches regarding the phylogeny of coronaviruses identified in cats''**presents a number of 220 pages, being structured as required in two parts: the first one containing the bibliographic study and the second one containing the original research. The first part entitled **„The present stage of knowledge"** contains 45 pages, 3 chapters, 23 subchapters, 15 figures. For its achievment 309 references were consulted.

Chapter I **„The description of the feline coronavirus"** contains 9 subchapters in which are presented facts about viral taxonomy, virus description, classification, theories regarding the virulent and avirulent strains, pathogenesis, epidemiological aspects, body reaction.

Feline coronavirus is classified in *Alphacoronavirus 1* genre, *Coronaviridae* family, *Nidovirales* order together with canine coronavirus and porcine transmissible gastroenteritis virus. The family name is due to the characteristic virion crown appearance when examined by electron microscopy.

Up to now there have been described two biotypes of feline enteric coronavirus and the feline infectious peritonitis, and two serotypes: I and II. Regardless of their classification in serotypes or biotypes there are benign coronavirus strains and highly virulent strains. Regarding the existence of the strains with different virulence there are several theories, the most famous being the internal mutation during replication of the feline enteric coronavirus which leads to feline infectious peritonitis virus.

The enteric coronavirus is usually easily spread between the individuals of the feline species causing mild symptoms, whereas FIP virus causes a fatal disease evolving with a low incidence. The enteric biotype has affinity to intestinal cells, while feline infectious peritonitis virus infects monocytes and macrophages. The infected body's defense mechanisms are ineffective since the disease is fatal.

Chapter II entitled **„Description of the clinical signs and anatomopathological signs in coronaviral infections"** is divided into five subchapters. This includes information from the specialty literature regarding the symptomatology in feline coronavirus infection determined by vasculitis lesions. Feline infectious peritonitis can evolve in several forms (wet or dry - acute or chronic), the histopathological pathognomonic lesions being represented by the pyogranuloma with various locations.

Chapter III, entitled **„The diagnosis in feline infectious peritonitis”** is divided into 9 subchapters, and presents the clinical and laboratory diagnostic methods. The X-ray and the ultrasound imaging methods are more frequently used compared to magnetic resonance. Haematological methods are used to observe changes in blood count and the presence of the nonregenerative anemia. Biochemical analyzes are carried out especially for blood samples and regards the evolution of the alpha and gamma globulin ratio. This methods can be performed on cerebrospinal fluid and effusions also.

The coronaviral antigen detection is achieved by molecular biology methods such as RT-PCR and real-time RT-PCR. For coronavirus antibodies identification in various pathological liquids serological methods are used. Most relevant methods for the FIP diagnosis are histopathology and immunohistochemistry achieved by marking macrophages with specific antibodies. Regarding the microscopic changes in FIP the characteristic lesion is pyogranuloma together with the presence of the vasculitis. Subchapters 8 and 9 relate the methods of treatment and specific and nonspecific prevention in FIP cases.

In the attempt to treat this disease there was used a variety of pharmaceutical substances including antivirals and interferons. These, however, have not been proven effective in feline infectious peritonitis. Until now no treatment plan by which affected individuals to succeed healing has been established. Therefore symptomatic and supportive treatment containing anti-inflammatory, antibiotics, immunomodulators drugs are recommended.

Regarding specific prophylaxis the feline coronavirus vaccines preparation trials have failed in most cases. There is a vaccine prepared on the basis of a heat-sensitive strain of coronavirus but this is not available in Europe, and its effectiveness was also disputed. The only way to defend against the coronavirus infections is to respect the nonspecific preventive measures. Avoiding stress, contact with the coronavirus eliminators, cleaning litter and food and water vessels, elimination from breeding of individuals coming from sensitive lines are effective methods to avoid transmission of infection.

The research part summarizes 150 pages, 73 figures and 25 tables, and includes eight chapters that presents the results concerning phylogenetic analysis of coronaviruses isolated from cats, and the examinations that led to them.

Chapter IV **„The aim and purpose of the research"** includes the objectives and the related activities required for the phylogenetic analyzes. It was necessary the selection of individuals from which various samples were collected and subjected to virological examinations.

This was achieved using the molecular biology techniques such as RT-PCR and real-time RT-PCR. Positive samples were then sequenced in order to obtain the encoded genetic

information. Based on these results the corresponding proteins necessary for the construction of phylogenetic trees have been simulated.

Chapter V entitled **„Clinical evaluation of cats suspected of feline infectious peritonitis”** includes three subchapters in which are presented the materials and methods, the results and discussion and the partial conclusions. The investigations aimed to identify coronavirus strains from carriers and eliminators with clinical signs of feline infectious peritonitis. Twenty-five cats from the Medical and Infectious Disease Clinics, Faculty of Veterinary Medicine casuistry and 11 cases from the private veterinary clinics across the city of Iasi were studied. 29 from the 36 cases were males and 6 were females, mostly around the age of two years.

For each case the medical history and clinical examination were performed using imaging methods such as ultrasound and radiography. For the effusion fluids with pathological appearance, the protein concentration was determined and cytology examination was performed.

The symptoms encountered in the examined cases have widely varied, frequently being observed behavioral changes, weight loss, kidney and digestive disorders, the accumulation of abdominal and / or thoracic fluids.

Chapter VI- **„Anatomical and histopathological aspects in cats with coronavirus infectious"** presents over two subchapters the macroscopic and microscopic changes in cats with infections caused by coronaviruses.

Subchapter 6.1. presents the anatomopathological results of the performed necropsies and the working methods. Ten corps were examined and in most cases the cahectic appearance and the presence of fluids and fibrin accumulated in the cavities was observed after their opening. Changes in the internal organs regarding in particular their volume have also been observed. A bacterial coinfection with a *Streptococcus spp.* was found in the case of a 2 years old male.

The most affected organs from the macroscopic point of view were the mezenteric lymph nodes, liver, spleen, kidneys. The changes were observed in the case of all cats examined.

In the parenchymal organs (kidney, liver) was observed the presence of white nodules determined by the antigen-antibody complexes.

In section 6.2. are presented the results of the histopathological examinations performed on slides from two individuals organs. The samples were embedded in paraffin and stained by HE and HEA methods. Microscopic lesions observed in examined organs were those of oedema, fibrin and cellular infiltration, necrosis, steatoses cases, vasculitis, hepatic and renal pyogranuloma, comparable to those mentioned in the literature as being typical for feline infectious peritonitis.

Chapter VII- **„Serological methods for coronaviral antibodies detection”** contains three subchapters in which the methods, results and partial conclusions are presented. Determination of coronavirus antibody titres was performed using indirect imunofluorescence technic for 12 blood and pathological fluids samples collected from FIP suspected cats and 8 blood samples coming from clinically healthy individuals. There were used PK cell line cultures infected with the porcine transmissible gastroenteritis virus. To highlight the antigen-antibody complexes direct imunofluorescence technic was used.

The serological examination performed on different pathological materials has shown a change in coronaviral antibody titer of 1/ 625 to 1/16000 for cats with clinical signs of feline infectious peritonitis and 1/125 to 1/3125 in clinically healthy cats.

Chapter VIII is entitled **„Virological examination using the real-time RT-PCR technique”**. The studied samples which consisted in pathological materials collected from FIP suspected cats and organs collected following necropsies are presented in this chapter. In order to perform the real-time RT-PCR technique the 205-211 pair of primers was required for amplification of a well preserved section in the *Coronaviridae* family genomes. As a positive control was used RNA derived from porcine transmissible gastroenteritis virus pure and 1/10 and 1/100 dilutions. Amplification was performed in a LighCycler from Roche, for each RNA analyzed sample being necessary pure and 1/10 diluted viral RNA.

Of the 47 analysed samples, the coronavirus was detected in 35 samples of feces, pathological fluids and organs. In the case of 4 cats taken into study all the samples collected from the intestine were positive, situation related to virus tropism for the enterocytes. The highest viral load has been registered in the case of a sample of the intestine, it became positive in a number of cycles (16.51) close to that of 1/100 dilution of positive control (15.65).

Chapter IX entitled **„Virological examination using the RT-PCR technique”** presents the methods and the results obtained after amplification of the studied samples using primers corresponding to 7b, 3c, M and N coronaviral genes. The samples were taken from both clinically healthy cats as well as from those suspected of FIP. Amplification was performed using the Qiagen One-Step RT-PCR commercial Kit and the results were visualised after electrophoresis in agarose gel. Positive samples were cut out from the gel, DNA being extracted using the MinEluteGel Extraction kit and sent for sequencing.

To characterize the identified coronavirus strains, RT-PCR has allowed getting 5 positive in 7b gene amplification, 23 positive results from M gene amplification, 27 positive results at 3c gene amplification, 28 positive results from N gene amplification.

Chapter X entitled **„Analysis / processing of the informations obtained by virological and sequencing methods”** contains 4 chapters and describes the working methods and the

results of the phylogenetic analysis. The sequencing results corresponding to each gene amplification were processed using *in silico* programmes to obtain proteins and the phylogenetic trees. The programmes used were accessed online on the website of the European Bioinformatics Institute (www.ebi.ac.uk), different versions of the software MEGA were used to obtain the trees.

The results of phylogenetic analysis showed that 7b coronavirale proteins identified in different organs of the same individual with FIP were related.

For the construction of phylogenetic trees based on matrix, canine and porcine coronavirus proteins strains were used. The strains which showed similarities with these were found in the samples of liver, pancreas and intestine collected from two individuals with FIP.

Using coronavirus strains from the specialty literature identified in healthy cats compared with the cats from our study and those with FIP from literature no segregation was observed between these for phylogenetic analysis based on N and 3c proteins. Following the membrane gene amplification three strains of coronavirus were found in the organ samples. These presented phylogenetic relations with coronaviruses identified in dogs which supports the classification among the viruses which exceed the species barrier. Five non-structural virtual proteins were obtained for 7b gene amplification, the phylogenetic analysis supporting the hypothesis that feline infectious peritonitis virus occurs as a result of mutations during viral replication.

Chapter XI includes **„The final conclusions”.**