SUMMARY

Key words: feline calicivirus, feline herpesvirus, cat, upper respiratory tract disease.

In our country, there is no report in literature regarding researches on feline calicivirus infection. This aspect, added to the importance of the disease in cats and to the fact that more and more people choose cats as pets, was the motivation of this doctoral thesis entitled: “Researches regarding feline calicivirus infection”.

The importance of feline calicivirus infection stems from its high prevalence, from the ease with which the virus is transmitted and from the fact that the disease is often associated to feline herpesvirus infection or to other bacterial diseases of the respiratory tract.

The work consists of 211 pages, being structured according to customs in two parts: the first part (45 pages) and the second part (113 pages), plus table of contents, introduction, summary and references.

The first part, “State of knowledge” is structured into 4 chapters synthesizing the main data in literature regarding feline calicivirus infection. The data presented are supported by 2 tables and 13 figures.

The second part, “Personal researches” consists of 7 chapters presenting purpose, objectives and research framework, the results of our investigations on viral infections of the upper respiratory tract of cats, the materials and working methods used, the interpretation of the results, conclusions and recommendations of the studies undertaken. The data obtained are recorded and illustrated in 10 tables and 65 figures.

For scientific documentation, 193 reference titles from national and international literature were used.

Upper respiratory tract disease are an important recurring problem for veterinarians and cat owners worldwide, and feline calicivirus and herpesvirus have been described as one of the main causes of infections.

Thus we set off to elaborate studies and researches regarding feline calicivirus infection, the purpose of the thesis being to establish: the presence and prevalence of feline calicivirus infection; the epidemiological, clinical and morphopathological aspects in feline calicivirus infection; the correlation between feline calicivirus and herpesvirus infection; risk factors in feline calicivirus infection; a virological and serological diagnosis protocol in feline calicivirus infection.

To achieve the purpose of the thesis, we undertook a descriptive epidemiologic investigation in Iassy county between January 1st 2010 - June 1st 2013. The investigations consisted of: periodical observation, collection and processing of data, biological sample collection and laboratory examination.

The observational descriptive study regarding feline calicivirus infection took place in the county of Iassy in the Internal Medicine and Infectious Diseases Clinics of the Faculty of Veterinary Medicine, as well as in veterinary practices and animal rescue associations, and in cat colonies around human settlements.

Virological and serological testing were done in the Institute for Diagnosis and Animal Health in Bucharest.

Feline respiratory diseases complex involves a variety of pathogens, feline calicivirus and herpesvirus type 1 being considered responsible for most cases, followed by bacterial infections of the respiratory system.

To establish the proportion the two viruses contribute to upper respiratory tract disease in cats, both viruses were investigated.

598 domestic cats (*Felis catus*) were examined clinically during the study. 247 biological samples were taken from 102 cats, for laboratory examination.

Of the 102 cats included in the study, 6 died during the epidemiological investigation.

Sample collection was done from indoor cats, feral cats living in colonies and cats from animal rescue associations. We took biological samples from cats with clinical signs which could be attributed to feline calicivirus and/or herpesvirus infections, as well as from clinically healthy cats, to determine their possible state of carrier.

From the cats with clinical signs, we collected oropharyngeal, nasal, conjunctival secretions and blood, and from the healthy cats, we collected oropharyngeal swabs and blood.

From the 6 cadavers of the cats studied we harvested organs (lungs, tonsils, tongue, liver, intestines and kidneys).

Diagnosing feline calicivirus and/or herpesvirus type 1 infection was done by the technique of viral isolation on cell cultures. Confirming the identity of the isolated viruses was obtained by tests of specific identification of the viral genome (real time RT-PCR and real time PCR).

Feline calicivirus infection was present in the cats included in our study, regardless of their place of origin (indoor cats, feral cats living in colonies or cats from animal rescue associations).

A lower rate of feline calicivirus infection cases was seen in indoor cats (18,92%), while a higher rate of infection was seen in feral cats from animal rescue association and cats living in colonies (28,12% and 33,33% respectively), which proves that the prevalence of feline calicivirus infection varies according to the number of animal populating the same habitat.

Another aspect regarding the epidemiology of feline calicivirus infection was noticed during the investigation. Of the 27 cats presenting with feline calicivirus infection, 4 kittens had been imported from a neighboring country (three Persian kittens, age two months and 15 days, from the same mother; a Russian Blue cat, aged two months), with the risk that the virus be transmitted directly or indirectly from acutely infected animals to those sensitive. The data recorded showed that cross-border transport of cats, regardless of purpose, can play an important role in the epidemiology of feline calicivirus infection especially when all legal conditions required in these cases are not met.

Risk factors in feline calicivirus infection were represented by not observing immunoprophylaxis measures (96% of the cases were unvaccinated cats) and living in a community (74% of the cases were cats from colonies or animal rescue associations, where cats live in large groups with unstable composition).

Sensitivity to feline calicivirus infection was high in cats younger than one year of age (52% of the cases), and an age lower than three months can be a risk factor for feline calicivirus infection (4 kittens with feline calicivirus infection and one with calicivirus-herpesvirus co infection died during the epidemiological investigation).

We did not notice a predisposition to feline calicivirus infection according to sex (56% males, 44% females) or breed (59% European, 19% Siamese, 11% Persian, 7% Birman and 4% Russian Blue; we took into account the fact that the European breed is the most common)

History data showed that among the cats with feline calicivirus infection, 25 showed clinical signs, while two feline did not show clinical signs at the time of consult, and at the time

of sample collection respectively. This proves that in feline calicivirus infection, cats can be asymptomatic carriers and shedders of virus.

Ulcers were the main anatomical and clinical sign present in cats with feline calicivirus infection, locating mainly on the anterodorsal side of the tongue (66,67%), less often on the hard palate (4%) and the nose (4%).

Clinical signs frequent in feline calicivirus infection in cats are represented by: ocular and nasal discharge (62,96%, and 48,15% respectively), apathy (59,26%), loss of appetite (51,85%), fever (40,74%), sneezing (25,93%) and dyspnea (25,93%). Less often (≤14,81%) we see clinical manifestations such as: hypersalivation, halitosis, coughing, diarrhea, anorexia, keratoconjunctivitis and gingivostomatitis.

Acute arthritis, a clinical form causing limping in kittens infected with feline calicivirus, was not identified.

The lesional aspects, correlated with the clinical signs showed a high incidence of lingual ulcers in cats infected with feline calicivirus (80%). Macroscopic lesions seen in cats with feline calicivirus infection were also represented by: interstitial pneumonia (40%), catarrhal tracheobronchitis (20%), catarrhal rhinitis (20%), as well as by catarrhal enteritis (80%), a lesion rarely described in literature as being associated to feline calicivirus infection in cats.

Interstitial pneumonia was represented on histological examination by: hyperplasia of the alveolar coating; lympho-plasmacytic hyperplasia; cellular infiltrate of lymphocytes, plasmatic cells and macrophages in the alveolar septa.

Cats included in our study also had **feline herpesvirus infection type 1**. A lower rate of feline herpesvirus infection was seen in indoor cats (13,51%), while feral cats living in colonies and cats from animal rescue associations had a higher infection prevalence (27,27% and 31,25%, respectively).

The main clinical manifestations in cats with feline herpesvirus infection were nasal signs (75%) associated to ocular signs (54,17%). Nasal signs were represented by seromucous and mucopurulent, abundant and continuous discharge. Ocular signs were seromucous and mucopurulent, abundant discharge, as well as by serous, seromucous, mucopurulent and purulent conjunctivitis.

Clinical signs frequent in feline herpesvirus infection in cats were represented by: sneezing (54,17%), loss of appetite (41,67%), apathy (41,67%), fever (33,33%) and dyspnea

(29,17%). Less often (≤12,5%) were seen anatomical and clinical manifestations such as: coughing, keratitis, keratoconjunctivitis, symblepharon, anorexia, prostration, uveitis, photophobia, nasal ulcers, dysphagia, diarrhea.

In the cats under study, corneal ulcers were not identified as the anatomical and clinical signs characteristic of feline herpesvirus type 1 infection.

Macroscopic lesions seen in cats with feline herpesvirus infection were described as: keratoconjunctivitis, catarrhal rhinitis, catarrhal tracheitis and interstitial pneumonia.

Feline calicivirus contributed in a higher proportion to **upper respiratory tract disease in cats** compared to feline herpesvirus (26,47% and 23,53% respectively).

It is recommended that further studies should be undertaken investigating the bacteria involved in upper respiratory tract infections in cats, in order to determine the share they contribute to the feline upper respiratory tract disease (*Bordetella bronchiseptica*, *Chlamydophila felis*, *Mycoplasma spp*, but also *Staphylococcus spp.* and *Escherichia coli*).

Feline calicivirus can be associated to feline herpesvirus in upper respiratory tract disease in cats. Laboratory tests showed the presence of both viruses in one of the 10 samples analyzed for this purpose, which proved the existence of feline calicivirus-herpesvirus coinfection in one of the cats included in the study (a Persian kitten, aged two months and 15 days).

The results of the investigations showed that most clinical signs of feline calicivirus and herpesvirus infection coincide. Therefore, most of the clinical signs present in upper respiratory tract disease in cats can be attributed both to feline calicivirus and feline herpesvirus infection (nasal discharge, ocular discharge, apathy, loss of appetite, fever, sneezing and dyspnea).

However, in the cats under study we noticed two aspects that can orient the clinician's diagnosis towards one of the diseases or the other.

Lingual ulcers were characteristic of feline calicivirus infection (66,67% of the cats with feline calicivirus infection; 4,17% of cats with feline herpesvirus infection), while feline herpesvirus infection caused nasal and ocular diseases with a high degree of severity, especially in kittens.

The diagnosis of feline calicivirus and/or feline herpesvirus type 1 infection was made by the technique of viral isolation on cell cultures.

Viral isolation on cell cultures involved taking appropriate samples (nasal, conjunctival and oropharyngeal swab, organs), inoculating them on the CRFK (Crandell-Rees feline kidney) feline kidney cell line and periodically examining them to observe the cytopathic effect.

The cytopathic effect attributed to feline calicivirus manifested after about 12 hours from inoculation of biological samples on the feline kidney cell line, and 2 days after inoculation we could also see the cytopathic effect caused by feline herpesvirus type 1.

The cytopathic effect attributed to feline calicivirus consisted of degenerative changes and cell destruction, as a result of viral multiplication: small cells, with a slightly granulated aspect; cell rounding; a tendency towards cell aggregation in clusters; destruction of the cell layer. The cytopathic effect of feline herpesvirus was characterized by syncytia formation and presence of localized areas of infection (focal degeneration).

Feline calicivirus was isolated especially from oropharyngeal and conjunctival secretions (26,92%, and 24,39% respectively), but also from nasal secretions (5,77%), as well as from pulmonary tissue. Feline herpesvirus type 1 was isolated mainly from conjunctival and nasal secretions (39,02% and 23,08% respectively), but also from oropharyngeal secretions (1,28%).

The results obtained prove that an important aspect in the technique of viral isolation on cell cultures is represented by the type of samples used for diagnosis. To diagnose feline calicivirus infection, it is recommended to use oropharyngeal and conjunctival swabs, and the diagnosis of feline herpesvirus infection should be based on conjunctival and nasal swabs.

Confirming the identity of the viruses isolated was achieved through tests of specific determination of the viral genome.

The qualitative detection of the feline calicivirus (RNA-virus) and of the feline herpesvirus (DNA-virus) from the viral isolates was done by the real-time RT-PCR technique and real-time PCR analysis, with TaqMan type hydrolysis probes.

The results recorded showed that the real-time RT-PCR test is more sensitive and faster compared to the technique of viral isolation of the feline calicivirus. From a number of 20 samples taken into study, 13 generated a positive response through the technique of real-time

RT-PCR and only 10 had a positive response on the viral isolation technique. The real-time RT-PCR took almost two days, and viral isolation took approximately two weeks.

The “real time” PCR test is sensitive and specific for determining viral nucleic acids, while the technique of viral isolation on cell cultures demonstrates the presence of a viable virus, capable of replication.

During our laboratory investigations, we met the situation where a positive sample on isolation of feline calicivirus on the CRFK feline kidney cell line generated a positive response on real-time PCR testing. This led to identification of both viruses on the same sample, which proves the existence of a calicivirus-herpesvirus coinfection in one of the cats taken into study.

Therefore, a positive sample on isolation of the feline calicivirus on cell cultures does not necessarily imply it’s negativity on feline herpesvirus infection, this is why we recommend testing for the specific determination of the viral genome of both viruses involved in upper respiratory tract disease in cats.

In general, the serologic diagnosis in feline calicivirus infection is not helpful because serological test can not differentiate between infection and vaccination. However, knowing the titre of antibodies necessary to neutralize the feline calicivirus can be useful, in order to anticipate if the cats are protected against the disease or not.

The results obtained showed that the indirect ELISA immunoenzyme assay can be used especially as a method for monitoring the efficiency of vaccination for feline calicivirus infection in cats, and less as a method of diagnosis in feline calicivirus infection. A Birman cat who had been vaccinated and showed no clinical signs, presenting a feline calicivirus infection identified by the technique of viral isolation and confirmed by the real-time RT-PCR test, generated a negative response on indirect ELISA testing, proving that in feline calicivirus infection serological tests can not differentiate between infection and vaccination.

We also noticed the fact that cats having been ill are not protected throughout their life, which is why it is recommended to vaccinate against calicivirus infection. In 15 unvaccinated cats, showing clinical signs and presenting an infection with feline calicivirus, we detected during our investigations a titer of antibodies >90 which does not ensure necessary protection against feline calicivirus infection.

Knowing the titer of antibodies for neutralizing the feline calicivirus is necessary in order to anticipate if the cats are protected against the disease or not. Vaccination is the only sure and effective measure for protecting cats against the disease (5 cats with no clinical signs, which had

been vaccinated prior to consultation, presented a titer of antibodies >270 during our investigations, which ensured necessary protection against feline calicivirus infection).

In feline calicivirus infection, vaccination is the only sure and effective measure of protection against the disease, but it does not ensure protection against infection or the carrier state. A vaccinated Birman cat, showing no clinical signs, presented for feline calicivirus infection which was identified by viral isolation techniques and confirmed by real-time RT-PCR, although this cat had an antibody titer >270, considered protective.