IDENTIFICATION OF CORONAVIRAL ANTIBODIES AND CORONAVIRUS - SPECIFIC ANTIBODY COMPLEXES IN ASCITES FLUID OF CATS DIAGNOSTICATED WITH FELINE INFECTIOUS PERITONITIS

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ABSTRACT - Feline infectious peritonitis (FIP) is an infectious lethal cat diseases, prodused by a mutant feline coronavirus (feline infectious peritonitis virus), which is manifested in two clinical forms. Dry form may go unnoticed or be confused with other diseases. Wet form, however, evolve with ascites fluid accumulation, its appearance being correlated with end-stage of the disease. Research has pursued the efficiency of direct immunofluorescence test for the identification of coronavirus - anticoronavirus antibody complexes in ascites fluid. Nine ascites fluid samples, obtained from cats aged between 1.7 months and 13 years, diagnosed with feline infectious peritonitis, were analyzed. The antibody titers were assessed using indirect immunofluorescence on pig kidney (PK) cell cultures infected with TGEV, on three samples, titres ranging from 1/25 and 1/625. All nine ascites fluid samples tested by direct immunofluorescence for detection of coronavirus specific antibodies complexes were positive. In images obtained with UV microscopy, fluorescence being seen in the macrophages under the form of a ring arranged on the periphery of the cell membrane and fluorescence localized intracellularly, probably internalized immune complexes. The results lead us to recommend the use of this test for FIP rapid diagnostic.

Key words: Coronavirus; Peritonitis; Immunofluorescence; Ascites; Complex.

REZUMAT - Identificarea complexelor specifice coronavirus – anticorpi anticoronavirusali în lichidul ascitic la pisicile diagnosticate cu peritonită infectioasă felină. Peritonita infectioasă felină (PIF) este o boală letală a pisicilor, produsă de o mutantă a coronavirusului enteric felin (virusul peritonitei infectioase feline), care se manifestă sub două forme clinice. Forma uscată poate trece neobservată sau poate fi confundată cu alte maladii. Forma umedă, în schimb, evoluează cu acumularea lichidului ascitic,

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apariția acestuia corelându-se cu stadiu
al bolii. Cercetăriile au urmărit eficiența
testului de imunofluorescență directă,
utilizat pentru identificarea complexelor
coronavirus – anticorpi anticoronavirus în
lichidul ascitic. Au fost analizate noua probe
de lichid ascitic, obținute de la pisici cu
vârsta cuprinsă între 1,7 luni și 13 ani,
diagnosticate cu peritonită infecțioasă
delină. Titrul de anticorpi a fost evaluat,
utilizând imunofluorescența indirectă pe
culturi celulare renale de porc, infectate cu
TGEV, pe trei probe, titrul variind între 1/25
și 1/625. Toate cele nouă probe de lichid
ascitic, testate prin imunofluorescența
directă pentru depistarea complexelor
coronavirus – anticorpi antitocoronavirus
directă pentru depistarea complexelor
coronavirus – anticorpi specifice, au fost
pozitive, ceea ce ne indreptățește să
recomandăm utilizarea testului pentru
diagnosticul rapid în PIF. În imaginile
obținute la microscopul cu UV s-au observat
celule (macrofage) cu fluorescență dispusă
sub formă de inel la periferia membranei
celulare. De asemenea, au putut fi observate
celule la care fluorescența este localizată
intracelulare, probabil complexele imune
internalizate.

Cuvinte cheie: coronavirus; peritonită;
imunofluorescență; ascită; complexe.

INTRODUCTION

Feline infectious peritonitis (FIP) is a fatal
disease of cats caused by a
 coronovirus, feline infectious
peritonitis virus (FIPV), able to infect
domestic and wild cats of all ages,
although younger ones and those over
14 years seem to be most susceptible.
FIPV is a part of Coronavirus family that comprised two genera, 
Coronavirus and Torovirus,
displaying similarities in morphology, genomic organization, and gene
expression (Gorbalenya et al., 2008).
Regarding genetic and serological
properties, there are three phylogenetic groups inside
Coronavirus genus (Enjuanes et al., 2000). Feline coronavirus (FCoV) is a member of antigenic group I, beside human coronaviruses (HCoV) 229E and NL63, porcine transmissible gastroenteritis virus (TGEV) and canine coronavirus (CCoV) (Erles et
al., 2003; Snijder et al., 2003). FIPV is considered to be a very pathogen
variety of enteric feline coronavirus.

Characteristic for the wet form of the disease is the accumulation of
fluid in different cavities, according to the
affected blood vessel. Ascites fluid appearance is correlated with
disease. According to the
literature FIPV enters target
macrophage/monocytes, binds to the
cell surface, being internalized by a
clathrin and caveolae independent and
dynamin dependent endocytosis (Van
Hamme et al., 2008). Dewerchin and
coworkers (Dewerchin et al., 2008)
added and suggested that viral
antigen-antibody complexes in FIP
were not internalized through any of
the previously described pathways,
the process being independent from
phosphatases and tyrosine kinases, but
depending on serine/threonine
kinases.

Virological diagnosis lasts 48
hours and is very expensive. A faster
method of diagnosis would be
welcome. There are commercial kits,
but not very cheap and therefore a
tsimpler method would be more
efficient.

Since the ascites fluid may
contain viral antigens and specific
antibodies that can be detected as a
complex, research has pursued the
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possibility of highlighting them using direct immunofluorescence test.

MATERIALS AND METHODS

Research was carried out on samples from nine cats diagnosed with FIP aged 1.7 months to 13 years, seven being the common race, a Burmese and a Russian Blue. Regarding gender distribution, three were males and six females.

To highlight the complex coronavirus - anticoronavirus antibodies, were tested by direct immunofluorescence (DIF) nine peritoneal effusion samples, identification of feline coronavirus previously been accomplished by the RT-PCR.

To identify anticoronaviral antibodies, indirect immunofluorescence reaction (IIF) on pig kidney cell culture (PK) infected with TGEV and incubated 48 h at 37°C was used. Cells were fixed with ethanol, washed, after which dilutions of ascites fluid were added and incubated for 60 minutes at 37°C. After further washes, fluorescein isothiocyanate-conjugated goat anti-feline antiserum (Jackson Immunoresearch) was added and incubated for 60 minutes at 37°C. Fluorescence was observed using IX51 Olympus inverted microscope. The titer was expressed as the highest dilution (1:25, 1:125, 1:625, 1:3,125, 1:1,6000) at which fluorescence was detectable.

In order to identify coronavirus – anticoronaviral antibody complexes direct immunofluorescence reaction was used, ascites fluid was centrifuged at 3000 rpm for 10 minutes and of the cells deposit, a smear was done. After fixation for 10 minutes and washing with ethanol, fluorescein isothiocyanate-conjugated goat anti-feline antiserum (Jackson Immunoresearch) was added and incubated for 60 minutes at 37°C. Fluorescent complexes were observed using IX51 Olympus inverted microscope.

RESULTS AND DISCUSSION

Using direct immunofluorescence reaction, all nine samples peritoneal effusions were positive (Table 1), demonstrating that the animals were exposed to feline coronavirus.

The images obtained at the immunofluorescence revealed a lot of cells (macrophages) with fluorescence with ring shape arranged on the periphery of the cell membrane (Fig. 1). Also, there can be observed cells without external green ring, but with fluorescence inside, perhaps internalized complexes.

Three of these samples were previously tested by indirect immunofluorescence for antibody titer determination, being positive, with values of 1/25, 1/125 and 1/625 (Fig. 2, 3).

As you can see, the immunofluorescence reaction can be used for detection of specific antibodies, viral antigens or immune complexes. Given that ascites fluid is an inflammatory exudate, macrophages, target cells for feline infectious peritonitis virus are present, it can be considered an extremely precious material for pathological diagnosis. Also, abdominal effusion may present large amounts of antibodies, coupled as complex with the coronavirus.
Table 1 - Cases presented at consultation, diagnosed with FIP

<table>
<thead>
<tr>
<th>No. Crt.</th>
<th>Specie</th>
<th>Breed</th>
<th>Sex</th>
<th>Age</th>
<th>Anamnesis</th>
<th>Results of DIF</th>
<th>Results of IIF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Feline</td>
<td>Common</td>
<td>Male</td>
<td>10 years</td>
<td>FIP wet form: anorexia, fever, ascites fluid accumulation in the abdominal cavity</td>
<td>+</td>
<td>1/125</td>
</tr>
<tr>
<td>2</td>
<td>Feline</td>
<td>Common</td>
<td>Female</td>
<td>13 years</td>
<td>FIP wet form: ultrasound examination: fluid with increased cellularity, tumors on the stomach, pancreas, intestine, mesentry, fibrin, ascites fluid.</td>
<td>+</td>
<td>1/625</td>
</tr>
<tr>
<td>3</td>
<td>Feline</td>
<td>Birman</td>
<td>Female</td>
<td>10 years</td>
<td>FIP, wet form: fluid, with increased turbidity in the abdomen and chest, on X-rays were observed lung opacification areas, abdominal type breath.</td>
<td>+</td>
<td>1/25</td>
</tr>
<tr>
<td>4</td>
<td>Feline</td>
<td>Russian Blue</td>
<td>Female</td>
<td>2 years</td>
<td>FIP, wet form: accumulation of fluid in the abdomen and chest, associated with respiratory problems</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Feline</td>
<td>Common</td>
<td>Female</td>
<td>1,7 months</td>
<td>FIP, wet form: accumulation of fluid in the abdomen</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Feline</td>
<td>Common</td>
<td>Male</td>
<td>2 years</td>
<td>FIP, wet form: clinical respiratory signs, conjunctivitis, ascites fluid in large quantities, very filament, yellow P-8, 1g/dl</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Feline</td>
<td>Common</td>
<td>Female</td>
<td>2 years</td>
<td>FIP, wet form</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Feline</td>
<td>Common</td>
<td>Male</td>
<td>1,5 years</td>
<td>FIP, wet form: hepatitis, nephritis, ascites fluid in small quantity, very pale mucous</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Feline</td>
<td>Common</td>
<td>Female</td>
<td>5 months</td>
<td>FIP, wet form: anorexia, fever, altered echogenicity of liver with presence of nodules, swollen blood vessels, fluid filament in small quantity.</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>
It is known that is practical impossible to make difference between feline enteric coronavirus (FCoV) and feline infectious peritonitis virus (VPIF), because the latter is a mutant of the first, the conditions in which the mutation occurs being unknown, just suspected. But, only FIPV has the ability to replicate in macrophages.

The acquisition of macrophage tropism appears to be an essential step in the transformation of an FCoV to an FIPV and from a largely non-pathogenic and localized enterocyte pathogen to a highly virulent and systemic monocyte/macrophage pathogen. The relationship between virulence and macrophage/monocyte
tropism has been firmly established in the literature (Pedersen, 2009).

The presence and titre of serum anticoronavirus antibodies have no clinical value if they are not related with specific symptoms, but may raise questions if they are identified. Occurrence of ascites in cats is related to about 50% of them with the suspicion of feline infectious peritonitis evolution.

Perhaps, the method we described may be useful to shorten the period to confirm or refute the diagnosis of feline infectious peritonitis. It is very important for practitioners who must adopt a certain therapeutic behavior depending on the results.

**CONCLUSIONS**

Nine ascites fluid samples, obtained from cats with ages between 1,7 months and 13 years, diagnosticated with feline infectious peritonitis, were analyzed.

The antibody titers were assessed using indirect immunofluorescence on pig kidney cells infected with TGEV, in three samples, titres ranging from 1 / 25 and 1 / 625;

All nine ascites fluid samples tested by direct immunofluorescence for detection of coronavirus - specific antibodies complex on the surface or inside macrophages were positive.

The results lead us to recommend the use of direct immunofluorescence test for rapid diagnosis of the PIF.

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**REFERENCES**


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