ASPECTS REGARDING MOLECULAR TECHNICS TO ESTABLISH THE CARRIER AND ELIMINATORS OF FELINE CORONAVIRUS

HORHOGEA Cristina¹, Mihai CARP-CĂRARE¹, Sophie LE PODER², Cristina¹ RÎMBU, Cătălin CARP-CĂRARE¹

University of Agricultural Sciences and Veterinary Medicine Ion Ionescu de la Brad Iasi
1 Faculty of Veterinary Medicine, Microbiology – Immunology Department
2 Ecole National Vétérinaire d’Alfort, Laboratoire de Virologie UMR 1161 France
8 Mihail Sadoveanu Alley, Cod 700489, Iasi, Romania
rebegeacristina@yahoo.com

Abstract. This aim of this paper is to evaluate the presence of Coronavirus in cat faeces and blood samples in order to establish the carriers and eliminators of this virus. The great importance of this carrier state consists in the fact that the cats, specially the ones from the holdhouses and from the owners that have at list two cats in the same house, because the coronavirus is highly contagious, but also can suffer mutations and transformation in feline infectious peritonitis virus which is lethal.

We analysed samples from 30 healthy and with clinical signs cats and we observed that the virus was identified only in 4 faeces samples using molecular methods like viral ARN extraction and amplification using RT-PCR method.

KEY WORDS: coronavirus, cat, faeces, carrier

INTRODUCTION

Coronavirosis in dogs and cats is a common infection produced by feline coronavirus (FCoV) and canine coronavirus (CCoV). Coronaviruses are, beside toroviruses, a part of Coronaviridae family and they are split in 3 groups in function of the antigenic properties genome organization. Feline coronaviruses form, with pig transmissible gastroenteritis virus (TGEV), canine coronavirus (CCoV) and human respiratory coronavirus 229 E, group 1.

Virus transmitted in the faeces of carrier cats and dogs is believed to be responsible for maintaining the infection in dogs, but specially the cat populations (Addie D.D., Jarrett O., 2001, Foley and all, 1997, Herrewegh and all, 1997). Most of the cats suffer only by a benign diarrhea, but a small proportion of infected cats develops feline infectious peritonitis (FIP), a fatal condition produced by an inappropriate immune response to the virus. To diagnose both FIP and FCoV infection seems to be difficult, partly because the type of FCoV that is common in cats and cannot be detected by isolation in cell culture. In the diagnosis of FCoV infection, beside virus detection, the appearance of antibodies has proved to be useful to prevent the transmission of the virus and to eliminate the infection from households of cats (Gonon and others 1995).

The ARN Coronavirus genome can be detected using RT-PCR (Addie D.D., Jarrett O., 2001, Herrewegh and all, 1995) so the diagnostic can be improved identifying carriers of the virus and cats that are free of the infection. All the positive cases for the presence of the feline
coronavirus, even in the absence of any clinical sign, are important to be known because any of those cats can become infected with feline infection peritonitis virus.

MATERIALS AND METHODS

The researches were made between 2008 – 2009 in the Faculty of Veterinary Medicine and Virology Laboratory UMR 1611 Alfort, France. The pathogen material was represented by 30 faeces samples taken from domestic cat (18 females and 12 males), with ages between 11 months and 15 years, from different breeds, 30 blood samples taken on anticoagulant (EDTA). The samples were obtained from 20 healthy animals (without clinical signs) and from 10 animals with clinical signs (benign diarrhea). We must add that the samples were taken from cats who lived at least two in the same environment.

In case of faeces samples we had 2 possibilities: samples taken from litter trays and rectal swabs. Rectal swabs had the advantage to be less contaminated with faeces from other cats, but faecal samples had the advantage to be stored for further investigations. In all cases we preferred the samples taken with rectal swabs.

All materials were tested using virusological methods: ARN extraction, amplification by RT-PCR using specific primers, DNA electrophoresis and analysing of amplified sequences using UV lamp.

The coronaviral ARN was extracted using Fluka RNA isolation kit and RNA plus Q-BIOgene, followed by RT-PCR using Qiagen one step. The primers that we used were represented by the couple 205/211 that allow the amplification of a not very specific but a very well conserved sequence of coronaviruses. A positive reaction means that the animal came in contact with a coronavirus group 1. More specific for the feline coronaviruses was the couple Mext 3/Mext 5 that code the synthesis of M protein.

RESULTS AND DISCUSSIONS

The aim of this study was to investigate the possibility of Coronavirus transmission from carrier cat to others from the same environment. We must say that all the cats that were examined came from at least 2 cats that lived in the same house. After the examination of the blood and faeces samples, coronaviral RNA was detected only in faeces came from 4 cats (fig. 1), revealing the fact that the animals were exposed to a coronavirus. Another observation is that all the blood samples tested seemed to be negative, so we conclude that none of the examined cats weren’t in a viremia state.

![Fig. 1 – Aspect of positive sample after RT-PCR and electrophoresis](image-url)
We thought in this protocole because, if the Coronavirus is highly contagious, but not very dangerous, the feline infectious peritonitis which apparently is a mutant Coronavirus, very harmful for animals and owners as well.

Most of the owners had at least 2 cats that use the same litter tray, the same food and water bottle, so the conditions for infection from one to another are fullfilled. In the case of cats that go outside, the sources on infection are multiple. So, we were interested to find out if the owners were in title to be afraid for their pets and the first aim was to find out if at least a part of the animals from different placed were carrier of a coronavirus to be able to put them in surveilance and the other animals, too.

For this first phase the blood and faeces samples were tested using as primers the couple 205/211 that allow to amplify a region of the viral genome very well conserved in coronaviruses.

All the tested blood samples were negative, but 4 of faeces samples were positive. In case to establish if there is a feline coronavirus we used another couple of primers (Mext 3/ Mext 5), that allow the amplification of genic sequences that code M membrane proteine and only 2 from the 4 positive samples were positive in this one too.

As it was observed in most of the epidemiological studies feline coronavirus may produce persistent infections. Often, cats may be carriers of Coronavirus but in many infections cases humoral or cellular immune system can’t fix the problem neutralising and eliminating the virus. Because any cat that carries any Coronavirus is potentially at risk for developing FIP, it is very important that those cat to be surveilled. However, cats with weak immune systems are most likely to develop the disease, including young and old cats. Most cats that develop FIP are under two years of age, but cats of any age may develop the disease.

Detection of FCoV in faeces using RT-PCR should be interpreted carefully because single positive or negative tests are meaningless as cats may shed intermittently or may be recently infected. As it was observed in literature datas to be identified as a chronic shedding carrier, a cat should be fecal virus positive on multiple tests over an 8-month period. A cat that tests negative on monthly tests over a 5-month period of time may be considered a non-shedder (Addie D.D., Jarrett O., 2001).

So, it is very important the cats to be examined and surveilled for the presence of at least feline or canine coronavirus in order to prevent the appearance of major problems.

REFERENCES


