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PANLEUKOPENIA OUTBREAK DIAGNOSED BY REAL TIME qPCR IN A CATTERRY IN ROMANIA

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Abstract

Feline parvovirus is a very often found cat infection, especially if we have unvaccinated animals and sometimes the infection can occur even on vaccinated animals, as we will describe in our paper. Feline Panleukopenia have been diagnosed in a cattery using a very sensitive test for this disease- Real Time qPcr. Twenty-one Edta blood samples have been tested, from British Shorthair cats from a cattery. Most of the tested cats had mild enteric symptoms and no death was registered. In our paper we prove that even on vaccinated animals we can found Parvovirus outbreaks and a very rapid and sensitive way to establish the diagnostic is a Real Time qPCR detection.

Keywords: panleukopenia, Real Time qPCR, cats

1. INTRODUCTION

Feline panleukopenia, also called "feline distemper" or feline "plague", known since 1920 it is produced by a parvovirus that can infect all members of the Felidae family but also, according to Parvovirus Infections in Wild Carnivores by Ariane Steine et all. [1] we can find this type of infections in minks, ferrets, foxes and raccoons. The panleukopenia virus causes from mild to severe gastrointestinal symptoms and can also be the cause of a severe panleukopenia and death. The etiological agent of this disease is a 5.2kb DNA single strain Parvovirus with a predilect tropism to dividing cells like those of the intestinal crypts epithelium, ensuing in acute enteritis, hemopoietic tissue ensuing in panleukopenia, lymphoid tissues ensuing in lymphoid depletion and in utero fetus, ensuing in fetal loss of life or cerebellar hypoplasia.

Most of the affected animals are young kittens that are not vaccinated or improperly immunized. (M. Rămneanțu, [6]).

Free in the environment the Parvovirus it's rather resistant bodily elements and chemical substances (Truyen, [2]). In contaminated environments it can also continue to be infectious for months. (Parrish, [3]).

The virus may be passed on by the fecal and oral routes. It initially replicates in the tissues of the oropharynx, and then it distributes itself throughout the body via a cell-free viraemia [7].

Because the genome is a single-stranded DNA molecule, its replication can only take place in cells that are in the S-phase of the cell division cycle.

As a result, the spread of the virus is limited to tissues that are actively undergoing mitosis. (Truyen [4]).

The first stage in the process of viral DNA replication and a need for transcription is for the cell to produce DNA polymerases, which are needed by all autonomous parvoviruses. These polymerases create the complementary DNA strand. (Truyen, [5]). The aim of this paper is to report an outbreak of feline parvovirus infection in a cattery with a unknown immunological status regarding this virus, for which the diagnostic was made by Real Time PCR.

2. MATERIALS AND METHODS

Twenty-one samples consisting in Edta blood were analyzed by Real Time Pcr for detection of Parvovirus. The samples were extracted from twenty-one British shorthair cats, with different ages and sex and all having the same owner and indoor space living. All the patients were vaccinated with at least, one dose against Feline Parvovirus. Most of the cats had mild enteric symptoms.

The amplification products from all positive samples were confirmed as Feline Parvovirus genes by nucleotide sequence analysis, which were positive using PCR amplification for the Feline Parvovirus.

Sampling: All the samples were sent and labelled by the attending physician and were blood samples collected on EDTA. Each sample was submitted with identification data for each animal (age, breed and sex).

2.1. Real Time qPCR:

The RT qPCR is a very specific and precise method of diagnosis. It is based on chain amplification of a certain nucleotide sequence for each pathogen, using specific primers and enzymes.

For this case EDTA blood vials were received into the lab for RT-qPCR, in less than 48 hours after the sampling.
The extraction of the DNA was made manually from 200µl blood, using Qiagen kit, following the manufacturer's extraction protocol. The extract was eluted in 100 µl ultrapure water and stored at -80° C.
Specific primers for Feline Parvovirus and enzymes were used. The amplification of the DNA was accomplished using a LightCycler analyzer.

3. RESULTS AND DISCUSSIONS

From 21 blood samples, 15 were found to be positive for Parvovirus on RT- qPcr detection. The samples have been identified with numbers from 1-21.
All the positive cats had mild clinical signs, consisting in general in mild enteric symptoms and no death was registered.
The oldest cat was 4 years old at the moment of the study and the youngest 4 months. All the cats had at least one dose of a vaccine against Feline Panleukopenia in very different moments in time.
The Highest Ct registered was 18.90 (Figure 1) and the lowest 34.26. The CT are not strictly related with the intensity of clinical signs but it reflects the amount of the virus in biological sample

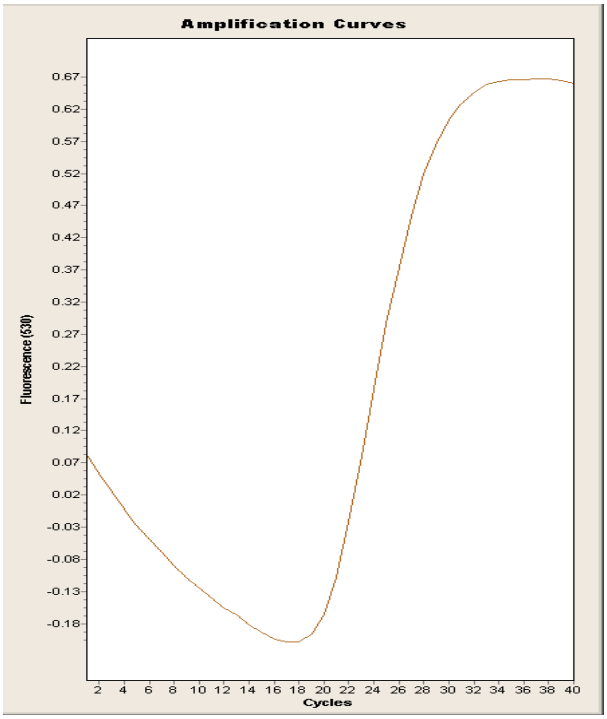


Figure 1. CT= 18.90 for cat no. 14

All the results from the RT-qPcr are displayed in Table nr 1.

Table 1. RT-qPCR results

Sample number	Age, sex	PCR Results
1	1y10m/F	31.42
2	1y9m/M	Negative
3	2y5m/F	34.26
4	1y6m/F	Negative
5	1y5m/F	34.0
6	2y1m/M	31.35
7	4m/F	Negative
8	4m/F	Negative
9	1y8m/F	Negative
10	4y/F	34.0
11	1y6m/M	34
12	1y8m/M	34
13	1y5m/M	Negative
14	1y2m/F	32.03
15	1y1m/F	Negative
16	8m/F	33.4
17	11m/F	18.90
18	11m/F	34
19	11m/F	32.57
20	11m/M	Negative
21	6m/F	Negative

(m= month. y= year. F= female.M= male)

4. CONCLUSIONS

Feline Parvovirus is a very often found disease in cats and it can affect even vaccinated animals, especially if immunizations have not been made according to protocols.

From twenty-one cats, twelve have been diagnosed as Feline Parvovirus infected by using the real time PCR method, meaning 57.14% of the tested samples

The RT -qPCR it is very useful for quick and precise diagnosis method of a disease like anaplasmosis, that has no pathognomonic appearance in her early stage. The amount of the sample needed it's only 200 µl of whole Edta blood and the result it's very specific.

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