Rapid Point-of-Care testing for feline calicivirus (FCV) using the portable isothermal loop-mediated nucleic acid amplification PLUSLIFE platform

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Laboratory Study Report: Evaluation of sensitivity and specificity of a POC - FCV test based on nucleic acid identification

System investigated: Pluslife Mini Dock (Guangzhou Pluslife Biotech, Guangzhou, China)

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1. INTRODUCTION

Cat upper respiratory tract infections (URIs), caused by a complex of highly contagious viral and bacterial pathogens, are particularly prevalent in feline populations, especially in high-density environments. The main pathogens associated with URI are feline calicivirus (feline calivicivirus: FCV), feline herpesvirus type 1 (feline heperpesvirus type 1: FHV-1) and *Chlamydia felis* (*C. felis*), while likely pathogens of secondary irruption are *Bordetella bronchiseptica* and *Mycoplasma felis*.

Feline calicivirosis, sustained by FCV (genus *Vesivirus*, family *Caliciviridae*), is a virtually worldwide disease characterized by respiratory symptoms and oral ulceration. The highest prevalence is found in feline colonies, especially in individuals aged 2 to 12 months. Persistence of FCV within the feline population can take place through direct contact between cats in the acute phase of disease and healthy cats, indirect contact resulting from virus resistance in the environment for several days to several weeks, and the establishment of long-term carrier status that follows clinical recovery. The clinical diagnosis of feline calicivirosis in its classical form is based on the finding of respiratory symptoms, but cats with respiratory symptoms may have a mixed infection, so the diagnosis of URI cannot be issued on the basis of clinical signs alone, as they are often overlapping. Therefore, specific laboratory tests aimed at identifying the pathogen must be used. The most widely used approach to date is that based on qualitative or quantitative RT-PCR (*Reverse Transcriptase-Polymerase Chain Reaction*) methods (*Real- Time RT-PCR*, qRT-PCR), which are commonly used to identify target RNA sequences from oro-pharyngeal, nasal, and conjunctival specimens, even with small amounts of virus, and are therefore also very useful in identifying asymptomatic carriers. In addition, etiologic diagnosis is also essential for rapid intervention with proper prophylaxis to prevent the spread of infection.

2. OBJECTIVE OF THE STUDY

In this report, we report the results of a preliminary study aimed at evaluating the sensitivity and specificity of a POC - FCV test based on the identification of FCV RNA using Pluslife Mini Dock device (Pluslife FCV Card).

3. MATERIALS AND METHODS

3.1 Sampling

Forty-seven samples of respiratory origin, represented by oro-pharyngeal or conjunctival swabs, from cats with symptomatology referable to URI were submitted for analysis. Each swab was resuspended in 1.5 ml of PBS (*Phosphate Buffered Saline*), frozen at -20°C and properly thawed at room temperature before each analysis. Samples were collected at the University Veterinary Teaching Hospital (OVUD) of the Department of Veterinary Medicine of the University of Teramo during the period from October 2022 to February 2024. At the time of sampling, an anamnestic survey form was completed for each cat under examination in which information regarding current symptomatology and any drug or vaccine treatment was collected.

3.2 Molecular screening by real time RT-PCR (qRT-PCR)

A total of 200 µl of PBS in which each buffer had been resuspended was subjected to nucleic acid extraction using ZymoBIOMICS MagBead DNA/RNA commercial kits (Zymo Research, USA). Each RNA extract (2.5 µl) was tested using a previously validated qRT-PCR procedure for FCV (Helps et al., 2002; Brunner et al., 2006). The qRT-PCR reactions, set up using the TaqMan™ Fast Virus 1-Step Master Mix (Applied Biosystem, Foster, CA, USA), were carried out in the "StepOne" thermal cycler (Applied Biosystem, Foster, CA, USA) under the thermal conditions given in the reference bibliography. A standard curve was generated using logarithmic dilutions from 10^{10} to 10^{0} copies per reaction, cloning the 83bp fragment of strain 160/2015/ITA (GenBank accession no. MT00824650) (Di Martino et al., 2020) obtained in qualitative RT-PCR, within the pCR 2.1 vector (TA Cloning Kit, Invitrogen, Ltd, Milan, Italy).

3.3 Analysis performed using the POC "FCV Nucleic Acid test (PLuslife FCV Card)" and Pluslife Mini Dock device

For analysis using the Pluslife Mini Dock device, each swab was resuspended in the lysis buffer provided by the FCV Nucleic Acid Test Card kit (Pluslife FCV Card), and the procedures specified by the manufacturer were performed.

The following Pluslife Mini Dock devices were used to perform the analyses:

- Device 1 Serial No.: 2247040335;
- Device 2 Serial No.: 2247040336.

3.4 Data analysis

The following parameters were calculated to evaluate the concordance between qRT-PCR results and the results obtained in the POC Pluslife test:

- *Positive Percent* Concordance (*Positive Percent Agreement-PPA*, sensitivity), calculated by evaluating the number of positive samples in qRT-PCR, which are also correctly detected as "positive" by the POC test;
- *Negative Percent* Concordance *Agreement-NPA* (specificity), calculated by evaluating the number of negative samples in qRT-PCR, which are also correctly detected as "negative" by the POC test;
- Overall diagnostic accuracy was calculated by determining the number of samples in which both analyses were in agreement.

4 RESULTS

FCV-specific qRT-PCR tests performed on the 47 samples showed 30 samples (63.8%, 30/47) as positive for FCV and 17 samples negative (17/47, 36.2%). Specifically, the positive samples showed *threshold cycle* (Ct) values ranging from 12.9 to 35.25 (mean Ct: 25.66), with viral loads ranging from 1.11×10^2 to 3.72×10^8 RNA copies/2.5 µl extract (mean 2.11×10^7 DNA copies/2.5 µl extract).

Analysis of the 47 samples performed with the Pluslife "FCV Nucleic Acid" POC test identified 30 (63.8%, 30/47) positive samples and 17 (36.2%, 17/47) negative samples.

The results thus obtained from the analysis with the Pluslife "FCV Nucleic Acid" POC test were compared with the results obtained in the screening performed with the specific qRT-PCR procedure.

In detail, it was found that of the 30 qRT-PCR positive samples, the Pluslife POC assay detected 28 positive samples with Ct values ranging from 12.9 to 35.25 (mean Ct: 25.30) and with viral loads ranging from 1.11×10^2 to 3.72×10^8 RNA copies/2.5 µl of extract (mean 8.58×10^7 RNA copies/2.5 µl of extract). Two samples (No. 29 and No. 30), with Ct of 31.8 and 29.4 and viral loads of 1.1×10^3 and 5.6×10^3 /2.5 µl of extract, respectively, were negative. Thus, the calculated sensitivity, expressed as PPA, was 93.3% (28/30). Of the 17 negative samples in qRT-PCR, 15 were also negative in the POC Pluslife test, so the calculated specificity, expressed as NPA, would be 88.2% (15/17), while 2 were positive.

The results of the tests performed by qRT-PCR and Pluslife "FCV Nucleic acid" POC test are shown in Table 1.

Table 1

CAT ID	PLUSLIFE DEVICE	qRT-PCR	
		Ct	Quantity
1	POS	30.77	2,25E+03
2	POS	17.48	1,71E+07
3	POS	29.75	4,46E+03
4	POS	20.42	2,37E+06
5	POS	24.97	1,11E+05
6	POS	20.17	2,80E+06
7	POS	35.25	1,11E+02
8	POS	23.13	3,83E+05
9	POS	25.15	9,84E+04
10	POS	23.98	2,16E+05
11	POS	33.1	4,69E+02
12	POS	31.94	1,02E+03
13	POS	24.47	1,56E+05
14	POS	29.42	5,57E+03
15	POS	27.17	2,53E+04
16	POS	25.94	5,79E+04
17	POS	31.93	1,03E+03
18	POS	23.65	2,70E+05
19	POS	18.26	1,01E+07
20	POS	29.78	4,38E+03
21	POS	21.35	1,29E+06
22	POS	26.81	3,22E+04
23	POS	25.08	1,03E+05
24	POS	13.65	2,25E+08
25	POS	25.95	5,75E+04
26	POS	31.0	1,93E+03
27	POS	12.9	3,72E+08
28	POS	25.19	9,59E+04
29	NEG	31.79	1,13E+03
30	NEG	29.4	5,65E+03
31	NEG	NEG	NEG
32	NEG	NEG	NEG
33	NEG	NEG	NEG
34	NEG	NEG	NEG
35	NEG	NEG	NEG
36	NEG	NEG	NEG
37	NEG	NEG	NEG
38	NEG	NEG	NEG
39	NEG	NEG	NEG
40	NEG	NEG	NEG
41	NEG	NEG	NEG
42	NEG	NEG	NEG

43	NEG	NEG	NEG
44	NEG	NEG	NEG
45	NEG	NEG	NEG
46	POS	NEG	NEG
47	POS	NEG	NEG

The overall diagnostic accuracy, calculated based on the number of samples in which both analyses agreed, was 91.5 percent (43/47), respectively.

5. DISCUSSIONS

In this study, the sensitivity and specificity of a POC - FCV test based on the identification of FCV RNA using Pluslife Mini Dock device was evaluated. For this purpose, the results obtained from the analysis of 47 respiratory specimens, collected from cats with clinical symptoms referable to URI, performed by FCV-specific qRT-PCR procedures and the results obtained from the analysis of the same by Pluslife Mini Dock device and "FCV Nucleic Acid" POC test (PLuslife FCV Card) were compared. The use of the latter allowed identification of FCV RNA in 28 of 30 swabs that tested positive in qRT-PCR, for which the sensitivity, understood as PPA, was 93.3%.

Considering specificity, understood as NPA, a value of 88.2% was calculated because of the 17 negative samples in qRT-PCR 15 were negative in the POC Pluslife test while 2 were positive. However, the negativity of these 2 samples was also confirmed by analysis with qualitative nested RT-PCR procedure (Marsilio et al., 2005).

6. BIBLIOGRAPHY

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