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9 **Barbara Di Martino, Camillo Di Rocco, Chiara Ceci, Fulvio Marsilio**

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18

19 **ABSTRACT**

20 To expand the epidemiological understanding of feline calicivirus (FCV) in dogs,  
21 genotypic and phenotypic characterizations of a FCV strain isolated from a puppy  
22 showing enteritis were performed. After isolation in cell culture, the novel isolate was  
23 analysed by RT-PCR and the amplicons obtained were sequenced. In order to  
24 characterize the growth properties of the isolate, the size of the plaques, the temperature  
25 of inactivation and the kinetics of growth were evaluated. Moreover, the novel strain was  
26 used to perform a serological study on 86 canine serum samples and 81 feline sera by  
27 virus-neutralization assay. The comparative analysis of nucleotide and amino acid  
28 sequences of the isolate, named FCV Te/10/07, revealed the highest identity to strain  
29 FCV-F65. The growth kinetic revealed that strain Te/10/07 grew more rapidly than F9  
30 strain.. By virus neutralization assay, dogs from the same region of the isolate showed  
31 antibodies against the FCV-F9 vaccinal strain in 63.9% (55/86) of sera, while antibodies  
32 against the Te/10/07 were found in 7 sera (8.13%). In cats neutralizing antibodies against  
33 Te/10/07 strain were recovered in 50.62% (41/81) of samples tested, even if 38 sera were  
34 positives for F9 strain with similar titres or higher. In three cats neutralization to Te/10/07  
35 alone was seen.

36 Our results confirmed the interspecific circulation of FCV strains among different animal  
37 species, but new investigations are needed to establish whether FCV is pathogenic in the  
38 dog and the role of interspecies circulation in pathogen spreading.

39

40 **KEY WORDS**

41 dog, feline calicivirus, isolation, characterization, serology

42

43 **INTRODUCTION**

44 Feline calicivirus (FCV), belonging to the family *Caliciviridae* genera *Vesivirus*, is a  
45 small non-enveloped virus with a diameter of 35-38 nm. The virus has a single-stranded,  
46 positive sense RNA genome approximately 7.7 kb in length and contains three open  
47 reading frames (ORFs): ORF1 (Sosnovtsev et al. 2002), ORF2 (Carter et al. 1992) and  
48 ORF3 (Sosnovtsev et al. 2005).

49 Although FCV isolates are closely related and constitute one serotype, there are several  
50 strains which differ in pathogenicity and antigenicity able to adapt in many  
51 heterogeneous conditions (Knowles et al., 1990).....

52 In cats, FCV infection is usually inapparent or associated with mild upper respiratory  
53 disease and/or signs of oral ulceration. Less commonly some isolates can cause an acute  
54 febrile lameness syndrome (Pedersen et al. 1983; Dawson et al. 1994) as well as abortion  
55 (Ellis 1981) and severe pneumonia. Moreover, during the last decade, highly virulent  
56 strains of FCV (VSD-FCV: Virulent Systemic Disease-FCV) have emerged that are  
57 associated with outbreaks of disease with high mortality (about 50%) (Pedersen et al.  
58 2000; Hurley et al. 2004; Pesavento et al. 2004; Coyne et al. 2006) and a new range of  
59 clinical features characterized by high persistent fever, severe edema of face and limbs,  
60 pancreatitis or pneumonia (Schorr-Evans et al. 2003).

61 FCV is a common pathogen of domestic cats, but has been isolated from several large  
62 felids in captivity and in the wild; for this reason it was suggested that all members of the  
63 *Felidae* are susceptible and [there are no alternative hosts (Radford et al. 2007)].

64 Occasionally some caliciviruses have also been found in dogs. Four apparently distinct  
65 types of calicivirus in dogs have been described: calicivirus serologically related to FCV  
66 (FCV-like) associated to glossitis or enteric infection (Evermann et al. 1981; Evermann et

67 al. 1985; San Gabriel et al. 1996; Pratelli et al. 2000a; Martella et al. 2002); canine  
68 calicivirus (CaCV), isolated from the feces of diarrheal dogs and which differed  
69 genetically and antigenically from FCV (Schaffer et al. 1985; Mochizuki et al. 1993);  
70 norovirus GIV.2 detected from a puppy with a severe gastroenteritis (Martella et al.  
71 2008); and calicivirus isolated from dogs with genital disease (Crandell 1988).  
72 A seroprevalence study (Pratelli et al. 2000b) has been shown that dogs FCV iare  
73 widespread in the dogs population, as well as in another report (Binn et al. 2000) has  
74 been observed an association between the presence of dogs and FCV infection in cats.  
75 However, to date, the role of these viruses in the epidemiology of FCV in cats and dogs is  
76 uncertain (Radford et al. 2007).

77 We report the results of a study based on genotypic and phenotypic characterization of a  
78 FCV strain isolated from a dog with enteritis and preliminary serological survey  
79 performed on a canine and feline population.

80

## 81 **MATERIALS AND METHODS**

### 82 *Virologic investigation*

83 One faecal sample collected from a three months-old dog with clinical signs of  
84 gastroenteritis was forwarded to our laboratories for virologic examination. Faecal  
85 sample was homogenised (10% w/v) in Dulbecco's Modified minimal essential medium  
86 (D-MEM) plus antibiotics (penicillin 5000 IU/ml, streptomycin 2500 µg/ml,  
87 amphotericin B 10 µg/ml), filtered and inoculated onto confluent Madin-Darby canine  
88 Kidney (MDCK), feline Crandell Kidney (CrFK) and dog adenocarcinoma (A72) cell  
89 line. After 3 days of incubation at 37 C, the inoculated cells were tested for FCV, canine  
90 coronavirus (CCoV) and canine adenovirus (CAV) antigens by Indirect  
91 immunofluorescence (IF) assay using a cat serum positive for FCV vaccinal strain F9, a

92 dog serum positive for CCoV and a dog serum positive for CAV antibodies. Faecal  
93 sample was passaged three times considered to be negative for, CCoV and CAV. The  
94 supernatant of the faecal homogenate and the inoculated monolayers were also subjected  
95 to hemoagglutination test (HA) using a suspension containing 1% pig erythrocytes and 1%  
96 foetal calf serum (FCS) in a 96-well V-plate to screen against canine parvovirus type 2  
97 (CPV-2). Results were read after 4 h at +4 C and expressed as the reciprocal of the  
98 highest sample dilution able to produce HA.

99 Faecal sample was also tested for type A rotavirus antigens by an Enzyme-linked  
100 immunosorbent assay (ELISA) (Biox Diagnostics, Jemelle, Belgium).

101

#### 102 *Molecular identification*

103 Viral RNA from the original faecal sample and infected cells was extracted using the  
104 QIAamp RNeasy Mini Kit (Qiagen GmbH, Hilden, Germany), according to the  
105 manufacturer's instructions. Template RNAs were eluted in 50 µl of RNase-free water  
106 and stored at -70 C until their use. DNA was extracted from faecal homogenate by  
107 boiling for 10 min and chilling on ice, whereas nucleic acid purification from infected  
108 cells was achieved using the DNeasy Tissue Kit (Qiagen GmbH, Hilden, Germany),  
109 followed by final elution of DNA with 200 µl of AE buffer and storage at - 70 C.

110 Viral RNA was analysed by reverse transcription (RT)-PCR using the following primer  
111 sets:

- 112 - Cali1-Cali2 (Marsilio et al. 2005) targeted to a region of the ORF2 gene of FCV;
- 113 - CaCV1-CaCV2 (Roerink et al. 1999) to amplify the complete ORF2 gene of  
114 CaCV;

- 115 - p289-p290 (Jiang et al. 1999), a broadly reactive primer pair, targeted to highly  
116 conserved motives of the RdRp region of the polymerase complex of *Vesivirus*,  
117 *Lagovirus*, *Norovirus* and *Sapovirus* genera.
- 118 - CCV1, CCV2 and CCV3 (Pratelli et al. 2001) to amplify a segment of the gene  
119 encoding for transmembrane protein M of CCoV.

120 Viral DNA was analysed by PCR using primers *555/For-555/Rev* (Buonavoglia and  
121 others 2001) that amplify a 583 bp fragment of the capsid protein-encoding gene of CPV-  
122 2. RT-PCR and PCR assays were performed as previously reported (Jiang et al. 1999;  
123 Roerink et al. 1999; Buonavoglia et al. 2001; Pratelli et al., 2001; Marsilio et al. 2005).  
124 The PCR products were analysed on agarose gel electrophoresis, stained with ethidium  
125 bromide and visualized using UV light.

#### 126 127 *Sequence analysis and phylogeny*

128 PCR products obtained were purified from agarose gel using the commercial kit Qiaex  
129 Gel Extraction (Qiagen GmbH, Hilden, Germany) according to the manufacturer's  
130 instructions. The DNA was then used as template for direct sequencing (Eurofins MWG  
131 Operon, Ebersberg, Germany) using primers p289-p290 (Jiang et al. 1999), Cali1-Cali2  
132 (Marsilio et al. 2005) and specific primers designed according to an overlapping strategy.  
133 The sequences were assembled and analysed using the BioEdit software package version  
134 2.1 (Hall 1999) and the NCBI's (<http://www.ncbi.nlm.nih.gov>) and EMBL's  
135 (<http://www.ebi.ac.uk>) analysis tools. Phylogenetic and molecular analyses were  
136 conducted using Mega 3 program (Kumar et al., 2004). The nucleotide sequence data of  
137 ORF1 and ORF2 genes have been deposited in the EMBL/GenBank database under  
138 accession no. EU980609 and EU980610, respectively.

139

140 *Phenotypic characterization*

141 Phenotypic characterization was performed following the method described by Ossiboff  
142 et al. (2007). In particular, the growth properties of the novel isolate were analyzed by  
143 plaque assay, temperature inactivation experiments (for 30 min at 41.8, 46.2 and 52.2 °C)  
144 and by generation of single and multiple cycle growth curves [4, 8, 12, 24 h post-  
145 infection (p.i.) at multiplicities of infection (MOI) of 10 and 0.02 respectively].  
146 Characterization was done comparing the growth properties of the isolate with those of  
147 the F9 vaccine strain.

148

149 *Virus-neutralization (VN) assay*

150 Eighty-six canine serum samples and 81 feline sera collected during years 2006-2008,  
151 were tested for neutralizing antibodies against the new isolate and the FCV-F9 vaccine  
152 strain. The neutralization assays were performed as previously described (Povey 1974).  
153 Briefly, 50 µl of serial two-fold diluted serum and 50 µl of infectious culture medium  
154 containing 100 TCID<sub>50</sub> of the FCV-F9 strain were mixed and incubated for 1 h at 37 C.  
155 One hundred microliters (1x10<sup>5</sup> cell/ml) of CrFK cell suspension was then added to each  
156 well. The cultures were then incubated for 3 days at 37 C, 5% with CO<sub>2</sub>. The assay was  
157 performed three time for each serum.

158

159

160 **RESULTS**

161 Typical FCV cythopatic effect, was evident from the first passage on CrFK cell cultures.  
162 No growth was observed in cultured cells of canine origin. Results were negative for  
163 CPV-2, CCoV, CAV and type A rotavirus antigens. The isolate was identified as FCV by  
164 IF test using a cat serum anti-FCV (Fig. 1).

165 Furthermore, the faecal sample and the infected cells at the passage one and three, were  
166 screened for *Caliciviridae* family, FCV, CaCV, CCoV and CPV-2 by conventional RT-  
167 PCR or PCR. Results were negative for CCoV, CaCV and CPV-2. Caliciviruses were  
168 identified by using primers p289-p290 (Jiang et al. 1999), whereas the virus was  
169 characterized as FCV by using primers Cali1-Cali2 (Marsilio et al. 2005) specific for the  
170 FCV capsid gene (Fig. 2).

171 Nucleotide analysis of the PCR product (924 bp in length) obtained by using specific  
172 primers for FCV (Cali1-Cali2), confirmed that the virus was closely related to FCV. The  
173 novel isolate was designated FCV-Te/10/07.

174 A sequence of 1557-nucleotides (nt) of ORF1 gene, encoding partial proteinase-  
175 polymerase region (pro-pol) (corresponding at nt position 3176-4733 of FCV-F9 vaccinal  
176 strain: GenBank accession no: M86379) and 1834-nt of ORF2 gene encoding A, B, C, D,  
177 E regions of the VP1 capsid protein (corresponding at nt 5371-7260 of F9 strain), were  
178 determined. BLAST and FASTA analyses of the ORF1 sequence, revealed nucleotide  
179 identity of Te/10/07 to the FCV-F9 of 78.4%, while identity to CaCV/48 was equal to  
180 52.6%. The highest sequence match was found to the F65 strain (UK/1990) (81% nt  
181 identity) isolated from cat with lameness and oral disease (Glenn et al. 1999). Alignment  
182 of ORF2 sequences showed nucleotide identity of Te/10/07 to FCVs sequence available  
183 on GenBank database, ranged from about 71% to 80%, while the identity to the F9 strain  
184 was 75%. The phylogentic tree based on the capsid amino acid sequence confirmed that  
185 the novel isolate clustered with the FCV group (Fig. 3).

186 Comparative analysis of the deduced amino acids (aa) sequences of the hypervariable  
187 region E (426-520 aa), revealed the highest similarity to strain LS015 (92%) (80% nt  
188 identity) (UK, 1985-1988) associated with chronic stomatitis (Glenn et al. 1999) and to

189 strain F65 (91.5%) (79% nt identity) (Glenn et al. 1999); similarity to FCV-F9 was about  
190 87% (75% nt identity), whereas to the canine strain 213/95 (Italy/2000) (Pratelli et al.  
191 2000a; Martella et al. 2002) was 84% (73% of nucleotide identity).

192 Investigation on the growth properties showed that the Te/10/07 strain tended to produce  
193 larger plaques (Fig. 4) than FCV-F9 in CrFK cells. Moreover, consistent with their larger  
194 plaque size, we found that the growth kinetic of the novel isolate was faster than F9  
195 strain. As described in Tab. 1, during the single-cycle growth kinetic experiment (MOI:  
196 10) we observed that the peak yield of FCV-Te/10/07 strain was produced between T1  
197 (4h p.i.) and T2 (8h p.i.), while FCV F9 strain peak yield occurred at T3 (12h p.i.) and T4  
198 (24h p.i.). In the multiple-cycle growth kinetic (MOI: 0.02) we found that the titres of  
199 Te/10/07 increased exponentially by 4h p.i. In contrast the F9 vaccine strain showed an  
200 exponential growth by 8-12 h p.i.

201 In thermal inactivation experiments we observed that the loss of infectivity was similar  
202 for both strains analyzed following 30 min incubation at 46.2 C and full inactivation at  
203 52.2 C (data not shown).

204 By serological investigation, neutralizing antibodies were present against the strain F9 in  
205 63.9% (55/86) of dog sera with titres from 1:2 to 1:16; whereas antibodies anti-FCV-  
206 Te/10/07 were found in only seven sera (8.13%) all of which had concurrent  
207 neutralization antibodies to F9. A serum of these showed neutralizing antibodies titres  
208 against Te/10/07 higher than F9. However, none of the samples tested resulted positive  
209 only for the dog strain (Tab. 2).

210 As reported in Table 3, the prevalence of FCV-F9-antibodies in cats was 76.54% (62/81).  
211 Neutralizing antibodies against Te/10/07 strain were recovered in 50.62% (41/81) of

212 samples tested, and 38 sera were positives for F9 strain too, with similar titres or higher.  
213 In three cats antibodies were found only against the dog strain.

214

215

## 216 **DISCUSSION**

217 The high degree of genomic plasticity that characterizes FCV has contributed to the  
218 emergence of several variants (Radford et al. 2007), some of which highly transmissible  
219 and associated with severe clinical disease (Dawson et al. 1994; Geissler et al. 1997;  
220 Pedersen et al. 2000). A number of studies investigated the properties of FCV infection in  
221 cats, in contrast little is known about the occurrence and the epidemiology of this virus in  
222 dogs, probably because only a few strains have been isolated in cell culture (Evermann et  
223 al. 1981; Evermann et al. 1985; San Gabriel et al. 1996; Pratelli et al. 2000a).

224 In the present report, an isolate of calicivirus from a three months old puppy (Te/10/07)  
225 showing enteritis, submitted to genotypic and phenotypic characterization.

226 Sequence analysis of ORF1 and ORF2 genes showed that FCV-Te/10/07, is a member of  
227 genogroup I (Geissler et al. 1997; Glenn et al. 1999; Sato et al. 2002). As previously  
228 demonstrated for other FCVs (Ossiboff et al. 2007; Radford et al. 2007), our strain not  
229 form a distinct group based on geographic origin, host range or clinical signs.

230 Comparison of hypervariable region E to the available sequences revealed no consistent  
231 differences among Te/10/07 strain and those associated with the most characteristic  
232 disease. By BLAST and FASTA analysis of ORF1 and ORF2 genes, our isolate appeared  
233 to be most genetically related to the F65 strain (UK/1990), that has previously been  
234 demonstrated experimentally to induce a febrile lameness syndrome (Dawson et al. 1994;  
235 Glenn et al. 1999). This observation allowed us to hypothesize that Te/10/07 doesn't

236 arose from a recombinant event between two different FCV strains or between a FCV  
237 strain and a closely related calicivirus.

238 Investigation on the *in vitro* growth properties of the Te/10/07 strain revealed that our  
239 isolate had rapid growth kinetic compared to F9. As previously hypothesized (Ossiboff et  
240 al. 2007) it may represent a marker of increased virulence of FCVs *in vivo*.

241 In accordance with another study performed in Italy (Pratelli et al. 2000b), the serological  
242 investigation showed that FCV-F9 is widely recognized in dog populations with a  
243 prevalence of 63.9%. In contrast, the percentage of dogs with antibodies to Te/10/07 was  
244 very low (8.13%) and none of the samples tested were positive solely for Te/10/07.  
245 Similarly in cats, despite of the high prevalence of antibodies against FCV-F9 vaccine  
246 strain, only three samples were positive against the Te/10/07. These results suggest that  
247 FCV-Te/10/07 is less related antigenically than other isolates to the commonly used  
248 vaccine strain F9.

249 In conclusion, this study confirms the interspecific circulation of FCVs among dogs and  
250 cats. The presence of this novel virus into dog population raises some concerns about the  
251 role of FCVs in puppy gastroenteritis. Further investigations are needed to determine if  
252 FCV-Te/10/07 may be an important cause of diarrhea in dog and if domestic dogs may  
253 represent a potential source of infection for cats.

254

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257

258

259 **References**

- 260 Binns, S. H., Dawson, S., Speakman, A. J., Cuevas L. E., Hart, C. A., Gaskell, C. J.,  
261 Morgan, K. L., Gaskell, R. M., 2000. A study of feline upper respiratory tract  
262 disease with reference to prevalence and risk factors for infection with feline  
263 calicivirus and feline herpesvirus. *J. Feline Med. Surg.* 2, 123-133.
- 264 Buonavoglia, C., Martella, V., Pratelli, A., Tempesta, M., Cavalli, A., Buonavoglia, D.,  
265 Bozzo, G., Elia, G., Decaro, N., Carmichael, L., 2001. Evidence for evolution of  
266 canine parvovirus type 2 in Italy. *J. Gen. Virol.* 82, 1555–1560.
- 267 Carter, M. J., Milton, I. D., Turner, P. C., Meanger, J., Bennett, M., Gaskell, R. M., 1992.  
268 Identification and sequence determination of the capsid protein gene of feline  
269 calicivirus. *Arch. Virol.* 122, 223-235.
- 270 Coyne, K. P., Reed, F. C., Porter, C. J., Dawson, S., Gaskell, R. M., Radford, A. D.,  
271 2006. Recombination of feline calicivirus within an endemically infected cat  
272 colony. *J. Gen. Virol.* 87, 921-926.
- 273 Crandell, R. A., 1988. Isolation and characterization of caliciviruses from dogs with  
274 vesicular genital diseases. *Arch. Virol.* 98, 65–71.
- 275 Dawson, S., Bennett, D., Carter, S. D., Bennett, M., Meanger, J., Turner, P. C., Carter, M.  
276 J., Milton, I., Gaskell, R. M., 1994. Acute arthritis of cats associated with feline  
277 calicivirus infection. *Res. Vet. Sci.* 56, 133-143.
- 278 Ellis, T. M., 1981. Jaundice in a Siamese cat with in utero feline calicivirus infection.  
279 *Aust. Vet. J.* 57, 383-385.
- 280 Evermann, J. F., Bryan, G. M. & Mckeirnan, A., 1981. Isolation of a calicivirus from a  
281 case of canine glossitis, *Canine Pract.* 8, 36-39.
- 282 Evermann, J. F., Mckeirnan, A. J., Smith, A. W., Skilling, D. E., Ott, R. L., 1985.  
283 Isolation and identification of caliciviruses from dogs with enteric infections. *Am.*  
284 *J. Vet. Res.* 46, 218-220.
- 285 Gabriel, S., Tohya, Y., Mochizuki, M., 1996. Isolation of a calicivirus antigenically  
286 related to feline caliciviruses from feces of a dog with diarrhoea. *J. Vet. Med. Sci.*  
287 58, 1041-1043.
- 288 Geissler, K., Schneider, K., Platzer, G., Truyen, B., Kaaden, O. R., Truyen, U., 1997.  
289 Genetic and antigenic heterogeneity among feline calicivirus isolates from distinct  
290 disease manifestation. *Virus Res.* 48, 193-206.

- 291 Glenn, M., Radford, A. D., Turner, P. C., Carter, M., Lowery, D., Desilver D. A.,  
292 Meanger J., Baulch Brown, C., Bennet, M., Gaskell, R. M., 1999. Nucleotide  
293 sequence of UK and Australian isolates of feline calicivirus (FCV) and  
294 phylogenetic analysis of FCV strains. *Vet. Microbiol.* 67, 175-193.
- 295 Hall, T. A., 1999. BioEdit: a user-friendly biological sequence alignment and analysis  
296 program for Windows 95/98/NT. *Nucl Acids Symp Ser* 41, 95-98.
- 297 Hurley, K. E., Pesavento, P. A., Pederesen, N. C., Poland, A. M., Wilson, E., Foley, J. E.,  
298 2004. An outbreak of virulent systemic feline calicivirus disease. *JAVMA* 224,  
299 241-249.
- 300 Jiang, X., Huang, P. W., Zhong, W. M., Farkas, T., Cubitt, D. W., Matson, D. O., 1999.  
301 Design and evaluation of a primer pair that detects both Norwalk- and Sapporo-  
302 like caliciviruses by RT-PCR. *J. Virol. Methods.* 83, 145-154.
- 303 Knowles, J. O., Dawson, S., Gaskell, R. M., Gaskell, C. Y.; Harvey, C., E., 1990.  
304 Neutralisation patterns among recent British and North American feline  
305 calicivirus isolates from different clinical origins. *Vet. Rec.* 127, 125-127.
- 306 Kumar, S., Tamura, K., Nei, M., 2004. MEGA3: Integrated software for Molecular  
307 Evolutionary Genetics Analysis and sequence alignment. *Brief Bioinform.* 5, 153-  
308 163.
- 309 Marsilio, F., Di Martino, B., Decaro, N. & Buonavoglia, C., 2005. A novel nested PCR  
310 for the diagnosis of calicivirus infections in the cat. *Vet. Microbiol.* 105, 1-7.
- 311 Martella, V., Lorusso, E., Decaro, N., Elia, G., Radogna, A., D'abramo, M., Desario, C.,  
312 Cavalli, A., Corrente, M., Camero, M., Germinario, C. A., Bányai, K., Di Martino  
313 B., Marsilio, F., Carmichael, L. E., Buonavoglia, C., 2008. Detection and  
314 molecular characterization of a canine Norovirus. *Emerg. Infect. Dis.* 14, 1306-  
315 1308.
- 316 Martella, V., Pratelli, A., Gentile, M., Buonavoglia, D., Decaro, N., Fiorante P.,  
317 Buonavoglia C., 2002. Analysis of the capsid protein gene of a feline-like  
318 calicivirus isolated from a dog. *Vet. Microbiol.* 85, 315-322.
- 319 Mochizuki, M., Kawanishi, A., Sakamoto, H., Tashiro, S., Fujimoto, R., Ohwaki M.,  
320 1993. A calicivirus isolated from a dog with fatal diarrhoea. *Vet. Rec.* 132, 221-  
321 222.

- 322 Ossiboff, R. J., Sheh, A., Shotton, J., Pesavento, P. A., Parker, J. S., 2007. Feline  
323 calicivirus (FCVs) isolated from cats with virulent systemic disease possess in  
324 vitro phenotypes distinct from those of other FCV isolates. *J. Gen. Virol.* 88, 506-  
325 517.
- 326 Pedersen, N. C., Elliott, J. B., Glasgow, A., Poland, A., Keel, K., 2000. An isolated  
327 epizootic of hemorrhagic-like fever in cats caused by a novel and highly virulent  
328 strain of feline calicivirus. *Vet. Microbiol.* 73, 281-300.
- 329 Pedersen, N. C., Laliberte, L., Ekman, S., 1983. A transient febrile limping syndrome of  
330 kittens caused by two different strains of feline calicivirus. *Feline Pract.* 13, 26-  
331 35.
- 332 Pesavento, P. A., Maclachlan, N. J., Dillard-Telm, L., Grant, C. K., Hurley, K. F., 2004.  
333 Pathologic, immunohistochemical, and electron microscopic findings in naturally  
334 occurring virulent systemic feline calicivirus infection in cats. *Vet. Pathol.* 41,  
335 257-263.
- 336 Povey, R. C., 1974. Serological relationship among feline caliciviruses. *Infect. Immun.*  
337 10, 1307-1314.
- 338 Pratelli, A., Greco, G., Camero, M., Normanno, G., Buonavoglia C., 2000a. Isolation and  
339 identification of a calicivirus from a dog with diarrhoea. *New Microbiol* 23, 257-  
340 260.
- 341 Pratelli, A., Martella, V., Elia G., Terio E., Lavazza, A., Buonavoglia C., 2000b.  
342 Characterization of a calicivirus strain isolated from a pup with enteritis. In:  
343 Brocchi, E., Lavazza, A. (Eds). *Proc. Fifth Int. Congress of ESVV*, 196-197.
- 344 Pratelli, A., Tempesta, M., Greco, G., Martella, V., Buonavoglia, C., 2001. Development  
345 of a nested PCR assay for the detection of canine coronavirus. *J. Virol. Methods*  
346 84, 91-94.
- 347 Radford, A. D., Coyne, K. P., Dawson, S., Porter, C. J., Gaskell, R. M., 2007. Feline  
348 calicivirus. *Vet. Res.* 38, 319-335.
- 349 Roerink, F., Hashimoto, M., Tohya, Y., Mochizuki, M., 1999. Organization of the canine  
350 calicivirus genome from the RNA polymerase gene to the Poly(A) tail. *J. Gen.*  
351 *Virol.* 80, 929-935.
- 352 Sato, Y., Ohe, K., Murakami, M., Fukuyama, M., Furuhashi K., Kishikawa S., Suzuki Y.,  
353 Kiuchi A., Hara M., Ishikawa, Y., Taneno, A., 2002. Phylogenetic analysis of

- 354 field isolates of feline calicivirus (FCV) in Japan by sequencing part of its capsid  
355 gene. *Vet. Res. Commun.* 26, 205-219.
- 356 Schaffer, F. L., Soergel, M. E., Black, J. W., Skilling, D. E., Smith, A. W., Cubitt, W. D.,  
357 1985. Characterization of a new calicivirus isolated from feces of a dog. *Arch.*  
358 *Virool.* 84, 181-195.
- 359 Schorr-Evans, E. M., Poland, A., Johnson, W. E., Pedersen, N. C., 2003. An epizootic of  
360 highly virulent feline calicivirus disease in a hospital setting in New England. *J.*  
361 *Feline Med. Surg.* 5, 217-226.
- 362 Sosnovtsev, S. V., Belliot, G., Chang, K. O., Onwudiwe O., Green, K. Y., 2005. Feline  
363 calicivirus VP2 is essential for the production of infectious virions. *J. Virol.* 79,  
364 4012-4024.
- 365 Sosnovtsev, S. V., Garfied, M., Green, K. Y., 2002. Processing map and essential  
366 cleavage sites of the non-structural polyprotein encoded by ORF1 of the feline  
367 calicivirus genome. *J. Virol.* 76, 7060-7072.
- 368
- 369

370 **Figure and table captions**

371

372 **Fig. 1 – Indirect immunofluorescence analysis on CRFK cells.** The intracytoplasmatic  
373 fluorescence was revealed by using a cat polyclonal antiserum raised against  
374 FCV-F9 vaccinal strain. Magnification, X400.

375

376 **Fig. 2 - RT-PCR with 289/290 and Cali1/Cali2 primer-sets.** Line 1: marker (Promega  
377 1 kb DNA ladder); line 2: PCR product obtained using generic calicivirus primers  
378 (289/290) of 287 bp in size; line 3: amplicon obtained by specific FCV  
379 (Cali1/Cali2) primer set of 924 bp in size.

380

381 **Fig. 3 - Phylogenetic analysis of the capsid amino acid sequence of the Te/10/07.** Tree  
382 was generated by using the Neighbour-joining distance method. For each strain,  
383 the country and year of isolation and the GenBank accession number are  
384 indicated.

385

386 **Fig. 4 - Plaque assay of Te/10/07 and FCV-F9.** After incubation at 37 °C for 48 h in  
387 humidified 5 % CO<sub>2</sub>, the overlay was removed and CrFK cells were stained with 1  
388 % (w/v) Neutral Red solution.

389

390 **Table 1:** Single- (MOI: 10) and multiple-cycle (0.02) growth kinetics of FCV-F9 and  
391 Te/10/07

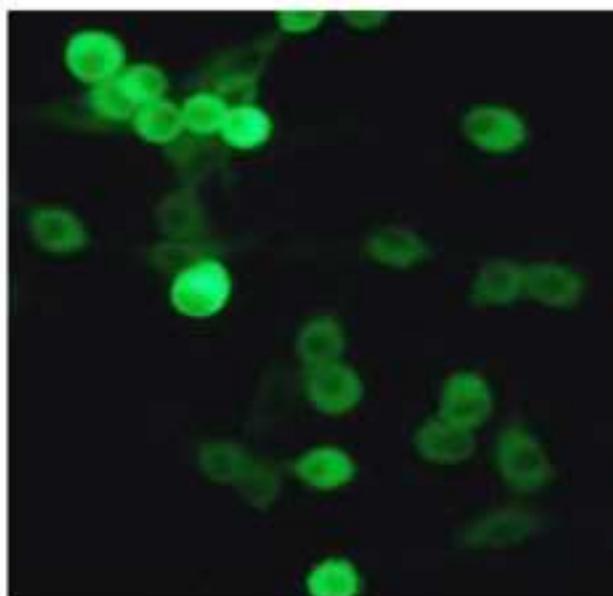
392

393 **Table 2:** Antibody titres determined by VN assay: the number of dog sera with each titre  
394 against FCV-F9 and 10-Te-07

395

396 **Table 3:** Antibody titres determined by VN assay: the number of cat sera with each titre  
397 against FCV-F9 and 10-Te-07

# FIG. 1



# FIG. 2

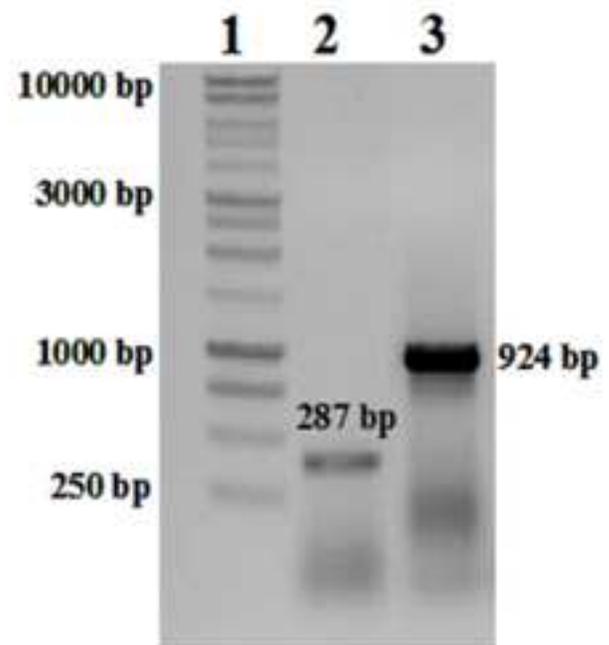
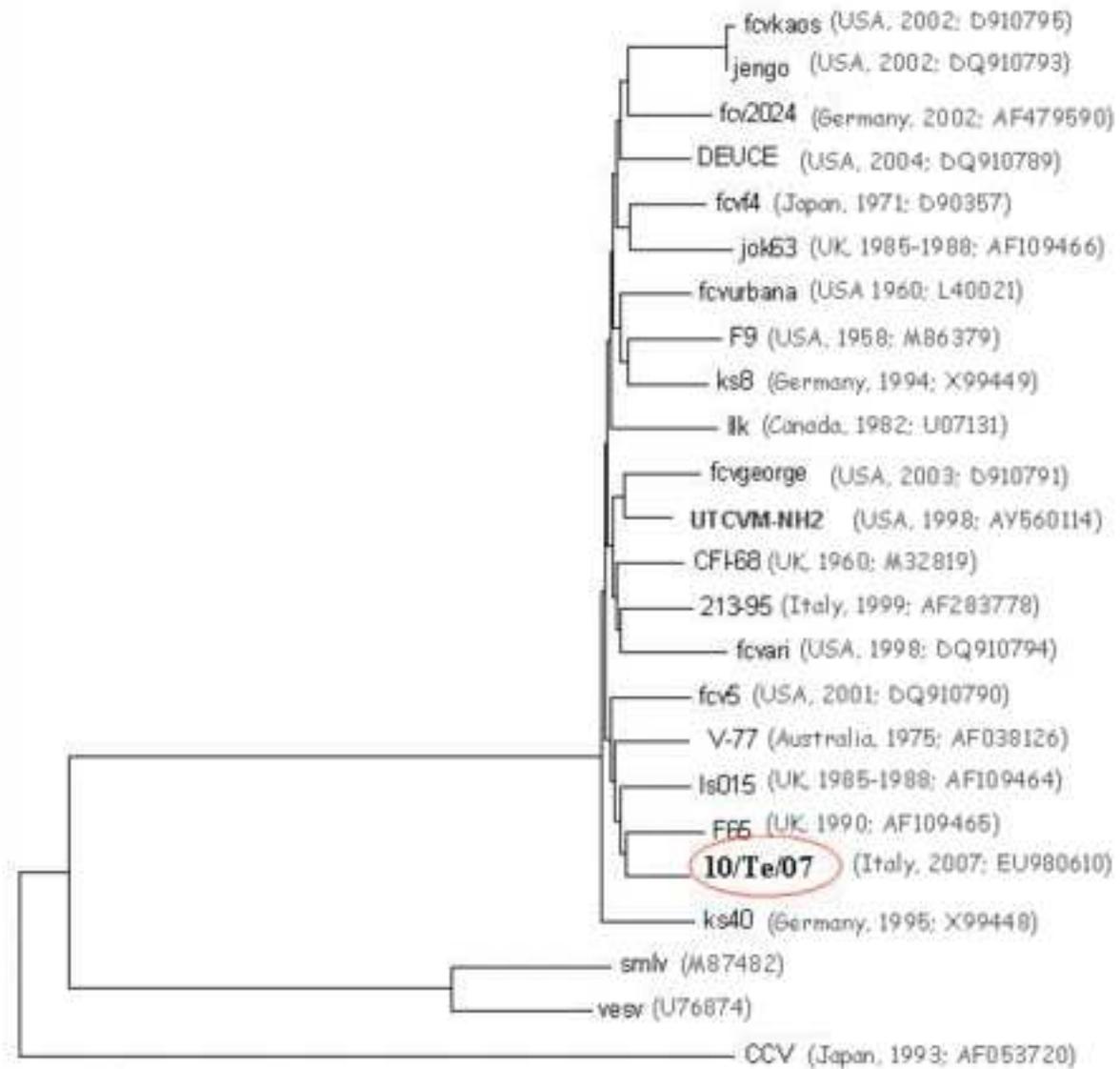


FIG. 3



trip

# FIG. 4

10/Te/07



FCV-F9



Table 1

	<i>MOI 10</i>				<i>MOI 0,02</i>			
	4h (T <sub>1</sub> )	8h (T <sub>2</sub> )	12h (T <sub>3</sub> )	24h (T <sub>4</sub> )	4h (T <sub>1</sub> )	8h (T <sub>2</sub> )	12h (T <sub>3</sub> )	24h (T <sub>4</sub> )
<b>FCV-F9</b>	1,2 x 10 <sup>7</sup>	1,6 x 10 <sup>7</sup>	4 x 10 <sup>7</sup>	3 x 10 <sup>6</sup>	1,4 x 10 <sup>6</sup>	1,2 x 10 <sup>7</sup>	1,1 x 10 <sup>8</sup>	2 x 10 <sup>7</sup>
<b>FCV-10/Te/07</b>	1 x 10 <sup>6</sup>	1,1 x 10 <sup>8</sup>	8 x 10 <sup>7</sup>	1,2 x 10 <sup>7</sup>	1,9 x 10 <sup>8</sup>	2 x 10 <sup>7</sup>	1 x 10 <sup>7</sup>	1,2 x 10 <sup>7</sup>

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Table 2

STRAINS	Positives							
	1:2*	1:4	1:8	1:16	1:32	1:64	1:128	1:256
FCV-F9	7	5	18	15	-	-	-	-
10/Te/07	-	1	2	4	-	-	-	-

\* serum dilutions

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Table 3

STRAINS	Positives							
	1:2*	1:4	1:8	1:16	1:32	1:64	1:128	1 $\geq$ 256
FCV-F9	6	3	2	35	1	1	2	12
10/Te/07	5	7	14	10	2	1	2	-

\*serum dilutions

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