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1 **An update on canine coronaviruses: viral evolution and pathobiology**

2

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19

20 **Abstract**

21 The emergence of human severe acute respiratory syndrome incited renewed interest in animal
22 coronaviruses (CoVs) as potential agents of direct and indirect zoonoses. The reinforced
23 epidemiological surveillance on CoVs has led to the identification of new viruses, genotypes,
24 pathotypes and host variants in animals and humans. In dogs, a CoV associated with mild enteritis,
25 canine coronavirus (CCoV), has been known since 1970s. CoV strains with different biological and
26 genetic properties with respect to classical CCoV strains have been identified in dogs in the last few
27 years, leading to a full reconsideration of the CoV-induced canine diseases. The genetic evolution
28 of dog CoVs is paradigmatic of how CoVs evolve through accumulation of point mutations,
29 insertions or deletions in the viral genome, that led to the emergence of new genotypes (CCoV type
30 I), biotypes (pantropic CCoV) and host variants (canine respiratory coronavirus). This paper is a
31 review of the current literature on the recent genetic evolution of CCoV and emergence of new
32 CoVs in the dog. The significances of the newly acquired information for the canine health status
33 and prophylaxis programmes are also discussed.

34
35 **Keywords:** dog; coronaviruses; genetic evolution; new genotypes/pathotypes.

36 **Coronavirus taxonomy and genomic organisation**

37 Coronaviruses (family *Coronaviridae*, order *Nidovirales*) are large, single-stranded, positive-sense
38 RNA viruses, which are responsible for enteric and/or respiratory disease in mammals and birds
39 (Enjuanes et al., 2000). Currently, CoVs are classified into three different antigenic groups,
40 although divergent CoVs have been identified in bats and wild carnivores in recent years, thus
41 suggesting revision of CoV taxonomy (Tang et al., 2006; Dong et al., 2007). Phylogenetic
42 relationship of CoVs of the different groups is represented in Fig. 1. Group 1 CoVs include canine
43 coronavirus (CCoV), feline coronaviruses (FCoVs) type I and type II, transmissible gastroenteritis
44 virus (TGEV) of swine, porcine respiratory coronavirus (PRCoV), porcine epidemic diarrhoea virus
45 (PEDV) and human coronaviruses 229E (HCoV-229E) and NL63 (HCoV-NL63). Recently, a ferret
46 coronavirus has been identified as a member of group 1 (Wise et al., 2006). Currently, group 2
47 CoVs are organised into bovine-like (subgroup 2a) and severe acute respiratory syndrome (SARS)-
48 like (subgroup 2b) viruses. Members of subgroup 2a are bovine coronavirus (BCoV), mouse
49 hepatitis virus (MHV), rat coronaviruses, porcine haemagglutinating encephalomyelitis virus
50 (PHEV), human coronavirus (HCoV) OC43, human enteric coronavirus (HECV) 4408 (Enjuanes et
51 al., 2000), and the newly recognised equine coronavirus (ECoV) (Guy et al., 2000), HCoV-HKU1
52 (Woo et al., 2005) and canine respiratory coronavirus (CRCoV) (Erles et al., 2003). SARS-CoV,
53 initially defined as prototype of a new group 4, has been placed more recently within group 2 CoVs,
54 in a subgroup 2b, together with SARS-like CoVs isolated from bats and wild carnivores
55 (Gorbalenya et al., 2004; Weiss and Navas-Martin, 2005). Group 3 comprises CoVs of avian origin,
56 whose prototype is represented by avian bronchitis virus, although the turkey coronavirus had been
57 placed previously in antigenic group 2 along with BCoV (Dea et al., 1990; Verbeek and Tijssen,
58 1991).

59 The 5' two-thirds of the 27.6-31-kb CoV genome consists of two overlapping open reading frames
60 (ORFs) that encode non-structural proteins including the viral RNA-dependent RNA polymerase
61 and proteases. Another one-third nucleotide sequences from the 3' end contain ORFs encoding for

62 the major structural spike, envelope, membrane, and nucleocapsid proteins. The trimeric spike (S)
63 protein, the main inducer of virus-neutralising antibodies (Gebauer et al., 1991), forms
64 characteristic viral peplomers which mediate viral attachment to specific cell receptors and fusion
65 between the envelope and plasma membrane (Enjuanes et al., 2000). The small membrane (E)
66 protein, recently recognised as a structural component of the coronavirions, is thought to be
67 important for viral envelope assembling (Vennema et al., 1996). The membrane (M) protein, the
68 most abundant structural component, is a type III glycoprotein consisting of a short amino-terminal
69 ectodomain, a triple-spanning transmembrane domain, and a long carboxyl-terminal inner domain
70 (Rottier, 1995). Antibodies to the M protein of MHV can neutralise viral infectivity, but only in the
71 presence of complement (Collins et al., 1982). The nucleocapsid (N) protein is a highly basic
72 phosphoprotein that modulates viral RNA synthesis, binds to the viral RNA and forms a helical
73 nucleocapsid (Enjuanes et al., 2000). Additional ORFs encoding non-structural proteins have been
74 recognised in CoV genomes and their number, nucleotide sequence and gene order can vary
75 remarkably among different CoVs (Boursnell et al., 1987; Lee et al., 1991; Herold et al., 1993;
76 Eleouet et al., 1995). The functions of such genes are in most cases unknown and most of them are
77 not essential for virus replication but may play a part in virulence and host range (Yamanaka et al.,
78 1998; Haijema et al., 2004). Group 2 CoV genomes contain an additional structural protein (HE)
79 with haemagglutinin-esterase activity, which shares up to 30% amino acid identity to the analogous
80 protein of influenza C viruses (Enjuanes et al., 2000).

81

82 **Canine enteric coronavirus (CCoV)**

83 ***History and pathobiology***

84 The first report on CCoV infection is dated 1971, when Binn and colleagues first isolated a
85 coronavirus (strain 1-71) from dogs with acute enteritis in a canine military unit in Germany (Binn
86 et al., 1974). The experimental administration of strain 1-71 to young dogs was able to reproduce
87 the gastroenteric disease (Keenan et al., 1976). Since then, several CCoV outbreaks have been

88 reported worldwide, showing that CCoV is an important enteropathogen of the dog. Serological and
89 virological investigations have demonstrated that CCoV is widespread in dog population, mainly in
90 kennels and animal shelters (Carmichael, 1978; Rimmelzwaan et al., 1991; Tennant et al., 1993;
91 Möstl et al., 1994; Bandai et al., 1999; Naylor et al., 2001b; Yeşilbağ et al., 2004; Schulz et al.,
92 2008). CCoV infection is characterised by high morbidity and low mortality, as well as by a typical
93 faecal-oral route of transmission (Tennant et al., 1991). CCoV is shed at high titres with the faeces
94 of the infected dogs and its infection is restricted to the alimentary tract, leading to the onset of
95 clinical signs typical of the gastroenteric involvement including loss of appetite, vomiting, fluid
96 diarrhoea dehydration and, only occasionally, death. Usually, systemic disease is not observed
97 during CCoV infection, although the virus has been isolated from several tissues (tonsils, lungs and
98 liver) of pups infected experimentally (Tennant et al., 1991). Fatal disease commonly occurs as a
99 consequence of mixed infections with CCoV together with canine parvovirus type 2 (CPV-2)
100 (Decaro et al., 2006, 2007b), canine adenovirus type 1 (Decaro et al., 2007a) or canine distemper
101 virus (Decaro et al., 2004a).

102

103 *CCoV genotypes*

104 Genetic analysis of several CCoVs detected in pups with diarrhoea in Italy revealed a number of
105 point mutations affecting a fragment of the M gene, which has led to the designation of these
106 atypical CCoVs as FCoV-like CCoVs (Pratelli et al., 2001). A genetic drift to FCoV type II was
107 also observed in the sequence of CCoVs detected in the faeces of two naturally infected pups during
108 the late stages of long-term viral shedding (Pratelli et al., 2002). Subsequently, extensive sequence
109 analysis on multiple regions of the viral genome, including ORF1a, ORF1b and ORF5, of several
110 CCoV positive faecal samples provided strong evidence for the existence of two separate genetic
111 clusters of CCoV. The first cluster includes CCoVs intermingled with reference CCoV strains, such
112 as Insavc-1 and K378, while the second cluster segregates separately from CCoVs and, presumably,
113 represents a genetic outlier referred to as FCoV-like CCoV (Pratelli et al., 2003b).

114 Finally, the nucleotide sequence of a region encompassing about 80% of the S gene of one of these
115 FCoV-like CCoV (strain Elmo/02) was determined (Pratelli et al., 2003a). Phylogenetic analysis
116 on the inferred amino acid sequence (Fig. 1) clearly showed that strain Elmo/02 segregates with
117 FCoVs type I (~ 81% identity) rather than reference CCoVs and FCoVs type II (~ 54% identity).
118 On the basis of the significant genetic similarity between Elmo/02 and FCoVs type I, this strain has
119 been designated as the prototype of the newly recognized CCoV type I, whereas reference CCoVs
120 have been referred to as CCoV type II (Pratelli et al., 2003a). Unlike group 1 CoVs, CCoV type I
121 shares with members of groups 2 and 3 a potential cleavage site in the S protein (Pratelli et al.,
122 2003a). Moreover, the genome of this genotype contains an additional ORF, 624 nt in length, which
123 has not been detected in CCoV type II and other group 1 CoVs (Lorusso et al., 2007, Fig. 2).
124 Computer-aided analysis of this additional ORF, which was referred to as ORF3, showed that the
125 putative encoded protein is 207 aa long, has a predicted molecular weight of about 24 kDa and an
126 isoelectric point of 7.02. Analysis of hydrophobic profile showed a neutral median hydrophaty
127 pattern with a highly hydrophobic region localised at the N-terminus due to the presence of a
128 leucin- and isoleucin-rich region. This region also contains a signal peptide with the aa cleavage site
129 at position 15 (12VAAKD16). This finding and the observation that no transmembrane region was
130 found suggest that the protein is secreted from the infected cells.

131 CCoV type I is distinguishable from CCoV type II by means of conventional RT-PCR assays,
132 which are able to amplify selectively fragments of the ORF2 and ORF5, but that genotype has not
133 been adapted to grow in vitro (Pratelli et al., 2004a). Recently, TaqMan-based real-time RT-PCR
134 assays have been established for detection and quantification of CCoV RNA in the faeces of dogs
135 with diarrhoea (Decaro et al., 2004b) and for discrimination between the two CCoV genotypes
136 (Decaro et al., 2005). Extensive molecular analysis of faecal samples collected from the Italian dog
137 population revealed that CCoV infection in dogs is frequently characterised by the simultaneous
138 presence of both genotypes (Decaro et al., 2005). The significance of the simultaneous infection by
139 both CCoV genotypes has to be determined, particularly with respect to the pathobiology of CCoV

140 infection, although failure to isolate CCoV type I on cell cultures hinders the acquisition of key
141 information on its pathogenic role in dogs.

142 Epidemiological investigations revealed that CCoV type I is now widespread in dogs in Turkey
143 (Yeşilbağ et al., 2004), Austria (Benetka et al., 2006) and China (Wang et al., 2006). In Austria,
144 CCoV type I-like M sequences were reported in cats (Benetka et al., 2006). In China, both CCoV
145 genotypes were also found in the faeces of healthy foxes and raccoon dogs, showing high genetic
146 relatedness with Italian canine isolates in the M gene (Ma and Lu, 2005; Wang et al., 2006). As for
147 the pathogenic potential of CCoV type I, the limited data available so far account for its
148 involvement in canine acute gastroenteritis as reported for CCoV type II. In fact, type I CCoVs
149 were detected in the faeces of dogs with diarrhoea after natural or experimental infection (Decaro et
150 al., 2005). Moreover, long-term viral shedding, up to 6 months, was reported in dogs naturally
151 infected (Pratelli et al., 2002).

152

153 ***Virulent/divergent strains***

154 Analogously to other CoVs, CCoV can mutate readily and new potentially virulent or genetically
155 divergent strains have been reported in the last few years. Sequence analysis of the S gene
156 sequences showed that some CCoV type II reference and field strains are more closely related to
157 TGEV than to FCoV (Wesseling et al., 1994; Horsburgh and Brown, 1995; Wesley, 1999). Naylor
158 et al. (2001a) identified a virulent strain (UWSMN-1) from an outbreak of fatal gastroenteritis in a
159 breeding colony in Australia, that appeared to be divergent from type II CCoVs circulating in other
160 countries. Sequence analysis of short genomic fragments showed nucleotide identities to reference
161 type II CCoVs up to 96.1%, 86.1% and 93.0% in ORF1b and in 5' and 3' ends of the spike gene,
162 respectively. Moreover, by phylogenetic analysis, strain UWSMN-1 was found to cluster separately
163 from typical canine and feline CoVs, indicating a gradual accumulation of mutations throughout its
164 genome rather than recombination events between CCoV and FCoV (Naylor et al., 2001a; 2002).

165 An epizootic outbreak caused by a hypervirulent strain of CCoV type II occurred in a beagle colony
166 in the United Kingdom (Sanchez-Morgado et al., 2004). The strain, isolate BGF10, was
167 characterised at molecular level, displaying an exceptionally long non-structural protein 3b (250
168 amino acids, Fig. 2) and a highly divergent N-terminus of the M protein.

169 Two cases of fatal CoV disease in pups without evidence of co-infection by CPV-2 were reported
170 by Evermann et al. (2005). CCoV infection was demonstrated by immunohistochemistry on gut
171 sections and electron microscopy of intestinal contents. Histopathology showed moderate depletion
172 and necrosis of lymphoid tissues, including thymus, spleen, lymph nodes and gut-associated
173 lymphoid tissues, in both pups. However, the authors did not conduct the genetic characterisation of
174 the CCoV strains detected, thus preventing any possible speculations on the molecular mechanisms
175 responsible for the exceptional strain virulence.

176 Further CCoV strains with high virulence were associated to a fatal outbreak of canine
177 gastroenteritis in Sweden (Escutenaire et al., 2007). The identification of different CCoVs was
178 highly suggestive of strains already circulating in the Swedish dog population rather than of new
179 emerging or imported variants. Importantly, some Swedish strains displayed an S gene with the 5'
180 and 3' ends closely related to CCoV type I and type II, respectively, thus indicating their possible
181 origin from recombination events between the two CCoV genotypes.

182

183 **Canine pantropic coronavirus**

184 In 2005, a highly virulent variant of CCoV type II (strain CB/05) was reported in Italy which caused
185 a systemic disease followed by a fatal outcome in pups (Buonavoglia et al., 2006). Clinical signs
186 consisted of fever (39.5-40°C), lethargy, loss of appetite, vomiting, haemorrhagic diarrhoea, severe
187 leukopenia and neurological signs (ataxia, seizures) followed by death within 2 days after the onset
188 of the symptoms. Necropsy examination revealed severe gross lesions in lungs, liver, spleen, and
189 kidneys. Virological and bacteriological investigations on the parenchymatous organs failed to
190 detect common canine pathogens, whereas CCoV type I and type II were identified in the intestinal

191 content of all pups by genotype-specific real-time RT-PCR assays. Unexpectedly, CCoV type II
192 RNA was also detected at high titres in lungs, spleen, liver, kidney and brain. A CCoV type II strain
193 (CB/05) was isolated on A-72 cells from all the examined tissues but brain. Immunohistochemistry
194 using a CCoV-specific monoclonal antibody detected CCoV antigen in all tissues. Sequence
195 analysis of the 3' genome end of the pantropic CCoV strain, including ORFs 2 (S gene), 3a, 3b, 3c,
196 4 (E gene), 5 (M gene), 6 (N gene), 7a and 7b, showed that strain CB/05 has a high degree of amino
197 acid identity to the cognate ORFs of CCoV type II, although the S protein displayed the highest
198 identity to FCoV type II strain 79-1683. A genetic marker was identified in the CB/05 genome,
199 consisting of a 38-nt deletion in ORF3b which was responsible for a predicted truncated non-
200 structural protein 3b (Decaro et al., 2007d, Fig. 2).

201 Experimental infection of seronegative pups with strain CB/05 reproduced the disease with
202 occurrence of severe clinical signs, including pyrexia, anorexia, depression, vomiting, diarrhoea and
203 leukopenia (Decaro et al., 2008a). A different clinical course was observed according to the age of
204 the infected pups. The older dogs, 6 months of age, slowly recovered from the disease, whereas two
205 out of three 2.5-month-old dogs were sacrificed due to the severity of the CB/05-induced disease.
206 The pantropism of the virus was confirmed by the presence of gross lesions in the internal organs of
207 the dead dogs, as well as by the detection of viral RNA in those tissues, including brains, albeit at
208 lower titres with respect to those detected in dogs succumbed to natural infection (Decaro et al.,
209 2007d). Traces of viral RNA were detected in the blood of a single dog, although further
210 unpublished studies have demonstrated that detectable RNemia (viral RNA in white blood cells)
211 can occur easily during CB/05 experimental infection (Decaro et al., unpublished).

212 In a subsequent experiment, strain CB/05 was proven to be able to infect even dogs with a recent
213 infection caused by an enteric CCoV strain, inducing the occurrence of mild clinical signs (Decaro
214 et al., manuscript in preparation). Although the dogs used in that study had a strong humoral
215 immunity to enteric CCoV at the time of challenge, experimental infection with strain CB/05 was
216 successful in all pups irrespective of the viral dose administered. Exposure to even low amounts of

217 virus would have similar infectivity on seropositive animals, since dogs inoculated with different
218 viral loads displayed the same duration of the viral shedding and not so very different viral titres in
219 the faeces. The duration of viral shedding was shorter and the clinical signs milder with respect to
220 previous observations in seronegative dogs (Decaro et al., 2007d), attributed mainly to the cross-
221 protection induced by antibodies against enteric CCoV. Lymphotropism of the strain CB/05 was
222 clearly demonstrated by the occurrence of moderate lymphopenia in several infected pups.
223 However, despite the moderate lymphopenia and the presence of the virus in the lymphoid tissues,
224 the viral RNA was not detected in the blood at any time. The association of strain CB/05 to a
225 severe, sometimes fatal, disease of dogs, together with the isolation of the virus from organs with
226 severe lesions, strongly suggests that CCoV has changed its tropism, acquiring the ability to spread
227 from the enteric tract to the internal organs (Decaro et al., 2007d). The molecular basis of the
228 change of virulence and tropism is being investigated through the assessment of a reverse genetics
229 system similar to that established for feline infectious peritonitis virus (Haijema et al., 2003).

230

231 **Canine respiratory coronavirus (CRCoV)**

232 As a consequence of the recent emergence of SARS-CoV and SARS-like viruses (Guan et al.,
233 2003), the role of CoVs as aetiological agents of novel diseases in the dog has been investigated. In
234 2003, a group 2 CoV was identified in the respiratory tract of dogs housed in a rehoming kennel in
235 the United Kingdom with a history of endemic respiratory disease (Erles et al., 2003). The viral
236 RNA was detected by RT-PCR in 32/119 tracheal and 20/119 lung samples, showing the highest
237 prevalence in dogs with mild clinical signs. The virus, referred to as canine respiratory coronavirus
238 (CRCoV), showed a close genetic relatedness to the bovine subgroup in the replicase and spike
239 proteins (Fig. 1). Sequence analysis of the S gene of CRCoV strain T101 revealed a nucleotide
240 identity of 97.3 and 96.9% to the group 2 CoVs BCoV and HCoV-OC43, respectively, suggesting a
241 recent common ancestor for the three viruses and demonstrating the occurrence of repeated host-
242 species shifts (Vijgen et al., 2005, 2006). An additional suggestion for the bovine origin of CRCoV

243 was provided by the successful experimental infection of pups with a typical BCoV strain
244 (Kaneshima et al., 2007). Conversely, CRCoV was found to be genetically unrelated to CCoV,
245 displaying only a 21.2% amino acid identity to the enteric virus in the S protein (Erles et al., 2003).
246 Unlike the enteric coronaviruses CCoVs type I and II, CRCoV is responsible for mild respiratory
247 signs and is recognised as aetiological agent of canine infectious respiratory disease (CIRD)
248 together with *Bordetella bronchiseptica*, canine adenoviruses type 1 and type 2, canine
249 parainfluenzavirus, canine herpesvirus, reoviruses and influenza viruses (Erles et al., 2004;
250 Buonavoglia and Martella, 2007). Due to the difficult adaptation of CRCoV to the in-vitro growth,
251 preliminary epidemiological surveys were carried out in the United Kingdom, North America,
252 Japan and Italy, by means of serological assays using the strictly genetically and antigenically
253 related BCoV as antigen (Erles and Brownlie, 2005; Priestnall et al., 2006; Kaneshima et al., 2006;
254 Decaro et al., 2007c). Those studies detected seropositivity rates comprised between 17.8%
255 (Kaneshima et al., 2006) and 54.7% (Erles and Brownlie, 2005). Serological evidence was obtained
256 that CRCoV has been circulating also in other countries, including Ireland and Greece (Priestnall et
257 al., 2006). Virological evidence for the CRCoV presence was provided for Canada, Japan and Italy.
258 In Canada, the CRCoV RNA was identified in archival tissue samples (both collected in 1996) from
259 2/126 cases of CIRD, but no genetic characterisation of the detected strains was performed (Ellis et
260 al., 2005). Further CRCoV strains were detected in the nasal (02/005) and rectal (04-009) swabs of
261 two Japanese dogs (Kaneshima et al., 2006). The nucleotide sequence identity of the Japanese
262 CRCoVs to reference T101 strain was 98.0-99.7% in the HE protein gene. The S gene was analysed
263 only for strain 02/005, showing a 99.1% identity to CRCoV-T101. The Italian survey found the
264 CRCoV RNA in a single lung sample out of 109 tested by RT-PCR, with a 98.0% sequence identity
265 to strain T101 in the S gene (Decaro et al., 2007c).

266 Although CRCoV has been detected in tissue samples of several dogs by RT-PCR, isolation of the
267 virus on canine cell lines as well as on the human adenocarcinoma cell line HRT-18 was at first
268 unsuccessful (Erles et al., 2003; Kaneshima et al., 2006). Only recently, Erles et al. (2006) were

269 able to propagate the CRCoV strain 4182 from a canine respiratory sample on HRT-18 cells and to
270 determine the full-length sequence of the 3' end of its genomic RNA (9.8 kb). By sequence
271 analysis, strain 4182 was found to have a genomic organisation similar to BCoV with a close
272 genetic relatedness to the bovine CoV subgroup in the major structural and non-structural proteins,
273 excepting for the ORFs encoding for small non-structural proteins between the S and E genes. In
274 that region, three different ORFs were identified in the BCoV genome, encoding for the non-
275 structural 4.9-kDa, 4.8-kDa and 12.7-kDa proteins, whereas only two ORFs, which encode for the
276 nonstructural 8.8-kDa and 12.8-kDa proteins, were present in the CRCoV genome. This mutation,
277 due to a 2-nt deletion prior to the stop codon that terminates the 4.9-kDa protein of BCoV leading to
278 the translation of a single 8.8-kDa joint protein, was found in all British CRCoVs but strain G9142.
279 Despite the multiple reports of CRCoV in different areas of the world, the role of CRCoV in CIRDC
280 is not completely clear. Analogously to other canine respiratory pathogens, it is likely that single
281 infections with CRCoV determine only a subclinical or asymptomatic course. However, CRCoV
282 replication in the respiratory epithelium may damage the mucociliary system, leading to the a more
283 severe clinical course of infections caused by other respiratory pathogens (Buonavoglia and
284 Martella, 2007).

285

286 **Epilogue**

287 Accumulation of point mutations, as well as small insertions and deletions in coding and non-
288 coding sequences, are the dominant forces in the microevolution of positive-sense RNA viruses,
289 resulting in proliferation of virus strains, serotypes and subtypes (Dolja and Carrington, 1992).
290 Extremely large (+) RNA virus genomes, such as those of CoVs, are thought to mutate at high
291 frequency as a consequence of high error rates of the RNA polymerase that are predicted to
292 accumulate several base substitutions per round of replication (Jarvis and Kirkegaard, 1991; Lai and
293 Holmes, 2001). Changes in virulence, tissue tropisms and/or interspecies transmission of CoVs
294 occur through genetic variations in structural and/or non-structural proteins (Laude et al., 1993;

295 Vennema et al., 1998; Guan et al., 2003; Rottier et al., 2005; Song et al., 2005; Vijgen et al., 2005;
296 Decaro et al., 2007d). The ORF2 of PRCoV has a 200-aa deletion in the N-terminus with respect to
297 TGEV, from which it presumably had arisen. Most likely, this deletion is responsible for the change
298 in the viral pathobiology (Vaughn et al., 1995). Nevertheless, minor amino acid differences in the
299 sequence of the spike protein have been shown to change the virulence of even very closely related
300 TGEV isolates (Sanchez et al., 1999). The enteric biotype of FCoV, feline enteric coronavirus
301 (FECV), causes persistent infections of the intestinal mucosa that may lead to point mutations in the
302 S gene (Rottier et al., 2005) and/or deletions in the group-specific genes 3c, 7b (Vennema et al.,
303 1998) or 7a (Kennedy et al., 2001). Those mutations have been suggested to be involved in changes
304 in the tropism of the virus, which may acquire the ability to infect monocytes/macrophages and to
305 cause a systemic, fatal disease of cats known as feline infectious peritonitis (FIP). Similar drastic
306 shifts of tissue tropism have been observed with murine coronaviruses (Haspel et al., 1978).
307 Adaptation to humans of the recently recognised SARS-associated coronavirus (SARS-CoV)
308 appears to be related to minor genome mutations, consisting of a 29-nt deletion in the genome of a
309 wild-mammal coronavirus, that resulted in the translation of two different ORFs, 10 and 11, instead
310 of the single ORF10 (Guan et al., 2003). There are multiple genetic and antigenic evidence that
311 several subgroup 2a CoVs, such as HCoV-OC43, HECV-4408 and PHEV, have arisen as
312 consequence of trans-species infections caused by BCoV (Zhang et al., 1994; Vijgen et al., 2005,
313 2006; Erles et al., 2006). Recently, bovine-like CoVs were identified in wild or domesticated
314 ruminants, including several species of deer, waterbuck antelope (Tsunemitsu et al., 1995), giraffe
315 (*Giraffa camelopardalis*) (Hasoksuz et al., 2007), alpaca (*Lama pacos*) (Jin et al., 2007), sable
316 antelope (*Hippotragus niger*) (Spiro et al., unpublished) and water buffalo (*Bubalus bubalis*)
317 (Decaro et al., 2008b) (Fig. 1), but the genetic determinants that may have caused the interspecies
318 transmission from cattle have not been identified so far.

319 Another important mechanism for CoV genetic evolution is the high-frequency of homologous
320 RNA recombination (Lai et al., 1985; Makino et al., 1986). This process is believed to be mediated

321 by a "copy-choice" mechanism (Cooper et al., 1974; Kirkegaard and Baltimore, 1986; Makino et
322 al., 1986). Recombination of CoV genomes has been observed during growth in tissue cultures (Lai
323 et al., 1985; Makino et al., 1986; Sanchez et al., 1999; Kuo et al., 2000), in experimentally infected
324 animals (Keck et al., 1988), and in embryonated eggs (Kottier et al., 1995). There also is evidence
325 for homologous recombination in IBV in the field (Jia et al., 1995). Recent findings suggest that
326 this mechanism also may be an important factor in the evolution of FCoV (Vennema et al., 1995;
327 Herrewegh et al., 1998).

328 In the few last years, CoVs of the dog have undergone a genetic evolution mainly through
329 accumulation of point mutations and deletions in some genomic regions rather than through
330 recombination events. Changes in the group-specific genes located between ORFs 2 and 4 have
331 been postulated to be responsible for increased virulence of CCoV type II strains (Sanchez-
332 Morgado et al., 2004; Decaro et al., 2007d). Point mutations in the S gene have been also suggested
333 to be involved in the emergence of pantropic CCoV (Decaro et al., 2007d). CRCoV emerged likely
334 as host variant of a BCoV strain that was able to spread from cattle to dogs (Erles et al., 2006). A
335 different origin could be hypothesised for CCoV type I. Based on the close genetic relatedness to
336 FCoV type I in the S gene, a potential recombinant origin of this CCoV genotype had been
337 suggested (Pratelli et al., 2003a). However, the recent identification of ORF3 in the genome of
338 CCoV type I should lead to reconsider its origin. In fact, since remnants of ORF3 were found in
339 some type II CCoVs, the most likely scenario is that CCoV type II has lost this gene during its
340 evolution, probably because ORF3 is not indispensable for viral replication (Lorusso et al., 2007).
341 Consequently, an ancestral carnivore CoV could be thought to have generated both CCoV
342 genotypes and FCoV type I as well.

343 The emergence of new CCoV genotypes and pathotypes in dogs poses intriguing questions on the
344 need for the development of specific vaccines prepared with the new virulent strains. Previous
345 studies demonstrated that inactivated vaccines currently used against enteric CCoV are poorly

346 effective, whereas an experimental modified-live virus (MLV) vaccine administered oronasally was
347 able to induce complete protection from disease as well as from infection (Pratelli et al., 2004c).
348 Preliminary data indicated that there is poor cross-reaction between CCoV types I and II at
349 serological level (Pratelli et al., 2004b) and that even the MLV vaccine does not prevent infection of
350 dogs after challenge with a CCoV type I strain (Buonavoglia et al., unpublished). However, the
351 lack of cell substrates supporting the in-vitro growth of CCoV type I hinders the development of
352 homologous vaccines prepared with the traditional technology, thus requiring innovative and
353 expensive systems, such as those used for production of recombinant vaccines. Moreover,
354 considering that strong immunity induced by natural infection with enteric CCoV was not able to
355 protect pups from challenge with pantropic CCoV (Decaro et al., manuscript in preparation), the
356 efficacy of currently used vaccines prepared with enteric CCoV strains is likely to be much poorer
357 against pantropic CB/05-like viruses. As for CRCoV, experimental vaccines should be developed
358 only if a clear pathogenic role in the occurrence of CIRD is demonstrated by future studies.

359 Further investigations would provide new insights into the molecular mechanisms responsible for
360 the change in viral pathobiology and into the pathogenic and immunological aspects of the canine
361 CoVs. At the same time, enduring epidemiological surveillance will help a timely identification in
362 dogs of further CoV strains with different genetic and biological properties and a more in-depth
363 comprehension of the pathogenic potential of these animal CoVs.

364 **References**

- 365 Bandai, C., Ishiguro, S., Masuya, N., Hohdatsu, T. and Mochizuki, M., 1999. Canine coronavirus
366 infections in Japan: virological and epidemiological aspects. *J. Vet. Med. Sci.* 61, 731-736.
- 367 Benetka, V., Kolodziejek, J., Walk, K., Rennhofer, M., Möstl, K., 2006. M gene analysis of atypical
368 strains of feline and canine coronavirus circulating in an Austrian animal shelter. *Vet. Rec.*
369 159, 170-174.
- 370 Binn, L.N., Lazar, E.C., Keenan, K.P., Huxsoll, D.L., Marchwicki, R.H., Strano, A.J., 1974.
371 Recovery and characterization of a coronavirus from military dogs with diarrhea. *Proc.*
372 *Annu. Meet. U. S. Anim. Health Assoc.* 78, 359-366.
- 373 Bournsnel, M.E., Brown, T.D., Foulds, I.J., Green, P.F., Tomley, F.M., Binns, M.M., 1987.
374 Completion of the sequence of the genome of the coronavirus avian infectious bronchitis
375 virus.
376 *J. Gen. Virol.* 68, 57-77.
- 377 Buonavoglia, C., Decaro, N., Martella, V., Elia, G., Campolo, M., Desario, C., Castagnaro, M.,
378 Tempesta, M., 2006. Canine coronavirus highly pathogenic for dogs. *Emerg. Infect. Dis.* 12,
379 492-494.
- 380 Buonavoglia, C., Martella, V., 2007. Canine respiratory viruses. *Vet. Res.* 38, 355-73.
- 381 Carmichael, L.E., 1978. Infectious canine enteritis caused by corona-like virus: current status and
382 request for information. Baker Institute Laboratory Report, series 2, no. 9. James A. Baker
383 Insitute for Animal Health, Ithaca, N.Y.
- 384 Collins, A.R., Knobler, R.L., Powell, H., Buchmeier, M.J., 1982. Monoclonal antibodies to murine
385 hepatitis virus-4 (strain JHM) define the viral glycoprotein responsible for attachment and
386 cell-cell fusion. *Virology* 119, 358-371.
- 387 Cooper, P.D., Steiner-Pryor, S., Scotti, P.D., Delong, D., 1974. On the nature of poliovirus genetic
388 recombinants. *J. Gen. Virol.* 23, 41-49.
- 389 Dea, S., Verbeek, A.J., Tijssen, P., 1990. Antigenic and genomic relationships among turkey and
390 bovine enteric coronaviruses. *J. Virol.* 64, 3112-3118.
- 391 Decaro, N., Camero, M., Greco, G., Zizzo, N., Elia, G., Campolo, M., Pratelli, A., Buonavoglia, C.,
392 2004a. Canine distemper and related diseases: report of a severe outbreak in a kennel. *New*
393 *Microbiol.* 27, 177-181.
- 394 Decaro, N., Pratelli, A., Campolo, M., Elia, G., Martella, V., Tempesta, M., Buonavoglia, C.,
395 2004b. Quantitation of canine coronavirus RNA in the faeces of dogs by TaqMan RT-PCR.
396 *J. Virol. Methods* 119, 145-150.

- 397 Decaro, N., Martella, V., Ricci, D., Elia G, Desario C, Campolo M, Cavaliere N, Di Trani L,
398 Tempesta M, Buonavoglia C., 2005. Genotype-specific fluorogenic RT-PCR assays for the
399 detection and quantitation of canine coronavirus type I and type II RNA in faecal samples of
400 dogs. *J. Virol. Methods* 130, 72-78.
- 401 Decaro, N., Martella, V., Desario, C., Bellacicco, A.L., Camero, M., Manna, L., D'aloja, D.,
402 Buonavoglia, C., 2006. First detection of canine parvovirus type 2c in pups with
403 haemorrhagic enteritis in Spain. *J. Vet. Med. B Infect. Dis. Vet. Public Health* 53, 468-472.
- 404 Decaro, N., Campolo, M., Elia, G., Buonavoglia, D., Colaianni, M.L., Lorusso, A., Mari, V.,
405 Buonavoglia C., 2007a. Infectious canine hepatitis: An "old" disease reemerging in Italy.
406 *Res. Vet. Sci.* 83, 269-273.
- 407 Decaro, N., Desario, C., Elia, G., Campolo, M., Lorusso, A., Mari, V., Martella, V., Buonavoglia,
408 C., 2007b. Occurrence of severe gastroenteritis in pups after canine parvovirus vaccine
409 administration: a clinical and laboratory diagnostic dilemma. *Vaccine* 25, 1161-1166.
- 410 Decaro, N, Desario, C., Elia, G., Mari, V., Lucente, M.S., Cordioli, P., Colaianni, M.L., Martella,
411 V., Buonavoglia C., 2007c. Serological and molecular evidence that canine respiratory
412 coronavirus is circulating in Italy. *Vet. Microbiol.* 121, 225-230.
- 413 Decaro, N., Martella, V., Elia, G., Campolo, M., Desario, C., Cirone, F., Tempesta, M.,
414 Buonavoglia, C., 2007d. Molecular characterisation of the virulent canine coronavirus
415 CB/05 strain. *Virus Res.*, 125, 54-60.
- 416 Decaro, N., Campolo, M., Lorusso, A., Desario, C., Mari, V., Colaianni, M.L., Elia, G., Martella,
417 V., Buonavoglia, C., 2008a. Experimental infection of dogs with a novel strain of canine
418 coronavirus causing systemic disease and lymphopenia. *Vet. Microbiol.* 128, 253-260.
- 419 Decaro, N., Martella, V., Elia, G., Campolo, M., Mari, V., Desario, C., Lucente, M.S., Lorusso, A.,
420 Greco, G., Corrente, M., Tempesta, M., Buonavoglia, C., 2008b. Biological and genetic
421 analysis of a bovine-like coronavirus isolated from water buffalo (*Bubalus bubalis*) calves.
422 *Virology* 370, 213-222.
- 423 Dolja, V.V., Carrington, J.C., 1992. Evolution of positive-strand RNA viruses. *Semin. Virol.* 3,
424 315-326.
- 425 Dong, B.Q., Liu, W., Fan, X.H., Vijaykrishna, D., Tang, X.C., Gao, F., Li, L.F., Li, G.J., Zhang,
426 J.X., Yang, L.Q., Poon, L.L., Zhang, S.Y., Peiris, J.S., Smith, G.J., Chen, H., Guan, Y.,
427 2007. Detection of a novel and highly divergent coronavirus from asian leopard cats and
428 Chinese ferret badgers in Southern China. *J. Virol.* 81, 6920-6926.
- 429 Eleouet, J.F., Rasschaert, D., Lambert, P., Levy, L., Vende, P., Laude, H., 1995. Complete genomic
430 sequence of the transmissible gastroenteritis virus. *Adv. Exp. Med. Biol.* 380, 459-461.

- 431 Ellis, J.A., McLean, N., Hupaelo, R., Haines, D.M., 2005. Detection of coronavirus in cases of
432 tracheobronchitis in dogs: a retrospective study from 1971 to 2003. *Can. Vet. J.* 46, 447-448.
- 433 Enjuanes, L., Brian, D., Cavanagh, D., Holmes, K., Lai, M.M.C., Laude, H., Masters, P., Rottier, P.,
434 Siddell, S., Spaan, W.J.M., Taguchi, F., Talbot, P., 2000. Family Coronaviridae, in: van
435 Regenmortel, M.H.V., Fauquet, C.M., Bishop, D.H.L., Carstens, E.B., Estes, M.K., Lemon,
436 S.M., Maniloff, J., Mayo, M.A., McGeoch, D.J., Pringle, C.R., Wickner, R.B. (Eds.), *Virus*
437 *Taxonomy, Classification and Nomenclature of Viruses*. Academic Press, New York, pp.
438 835-849.
- 439 Erles, K., Toomey, C., Brooks, H.W., Brownlie, J., 2003. Detection of a group 2 coronavirus in
440 dogs with canine infectious respiratory disease. *Virology* 310, 216-223.
- 441 Erles, K., Dubovi, E.J., Brooks, H.W., Brownlie, J., 2004. Longitudinal study of viruses associated
442 with canine infectious respiratory disease. *J. Clin. Microbiol.* 42, :4524-4529.
- 443 Erles, K., Brownlie, J., 2005. Investigation into the causes of canine infectious respiratory disease:
444 antibody responses to canine respiratory coronavirus and canine herpesvirus in two
445 kennelled dog populations. *Arch. Virol.* 150, 1493-1504.
- 446 Erles, K., Kai-Biu, S., Brownlie, J., 2006. Isolation and sequence analysis of canine respiratory
447 coronavirus. *Virus Res.* 124, 78-87.
- 448 Escutenaire, S., Isaksson, M., Renström, L.H., Klingeborn, B., Buonavoglia, C., Berg, M., Belák,
449 S., Thorén, P., 2007. Characterization of divergent and atypical canine coronaviruses from
450 Sweden.
451 *Arch. Virol.* 152, 1507-1514.
- 452 Evermann, J.F., Abbott, J.R., Han, S., 2005. Canine coronavirus-associated puppy mortality without
453 evidence of concurrent canine parvovirus infection. *J. Vet. Diagn. Invest.* 17, 610-614.
- 454 Gebauer, F., Posthumus, W.A.P., Correa, I., Suñé, C., Sánchez, C.M., Smerdou, C., Lenstra, J.A.,
455 Meloen, R., Enjuanes, L., 1991. Residues involved in the formation of the antigenic sites of
456 the S protein of transmissible gastroenteritis coronavirus. *Virology* 183, 225-238.
- 457 Gorbalenya, A.E., Snijder, E.J., Spaan, W.J., 2004. Severe acute respiratory syndrome coronavirus
458 phylogeny: toward consensus. *J. Virol.* 78, 7863-7866.
- 459 Guan, Y., Zheng, B.J., He, Y.Q., Liu, X.L., Zhuang, Z.X., Cheung, C.L., Luo, S.W., Li, P.H.,
460 Zhang, L.J., Guan, Y.J., Butt, K.M., Wong, K.L., Chan, K.W., Lim, W., Shortridge, K.F.,
461 Yuen, K.Y., Peiris, J.S., Poon, L.L., 2003. Isolation and characterization of viruses related to
462 the SARS coronavirus from animals in southern China. *Science* 302, 276-278.
- 463 Guy, J.S., Breslin, J.J., Breuhaus, B., Vivrette, S., Smith, L.G., 2000. Characterization of a
464 coronavirus isolated from a diarrheic foal. *J. Clin. Microbiol.* 38, 4523-4526.

- 465 Haijema, B.J., Volders, H., Rottier, P.J., 2003. Switching species tropism: an effective way to
466 manipulate the feline coronavirus genome. *J. Virol.* 77, 4528-4538.
- 467 Haijema, B.J., Volders, H., Rottier, P.J., 2004. Live, attenuated coronavirus vaccines through the
468 directed deletion of group-specific genes provide protection against feline infectious
469 peritonitis. *J. Virol.* 78, 3863-3871.
- 470 Hasoksuz, M, Alekseev, K., Vlasova, A., Zhang, X., Spiro, D., Halpin, R., Wang, S., Ghedin, E.,
471 Saif, L.J., 2007. Biologic, antigenic, and full-length genomic characterization of a bovine-
472 like coronavirus isolated from a giraffe. *J. Virol.* 81, 4981-4990.
- 473 Haspel, M.V., Lampert, P.W., Oldstone, M.B., 1978. Temperature-sensitive mutants of mouse
474 hepatitis virus produce a high incidence of demyelination. *Proc. Natl. Acad. Sci. U.S.A.* 75,
475 4033-4036.
- 476 Herold, J., Raabe, T., Siddell, S., 1993. Molecular analysis of the human coronavirus (strain 229E)
477 genome. *Arch. Virol. Suppl.* 7, 63-74.
- 478 Herrewegh, A.A.P.M., Smeenk, I., Horzinek, M.C., Rottier, P.J.M., de Groot, R.J., 1998. Feline
479 coronavirus type II strains 79-1683 and 79-1146 originate from a double recombination
480 between feline coronavirus type I and canine coronavirus. *J. Virol.* 72, 4508-4514.
- 481 Horsburgh, B.C., Brown, T.D., 1995. Cloning, sequencing and expression of the S protein gene
482 from two geographically distinct strains of canine coronavirus. *Virus Res.* 39, 63-74.
- 483 Jarvis, T.C., Kirkegaard, K., 1991. The polymerase in its labyrinth: mechanisms and implications of
484 RNA recombination. *Trends Genet.* 7, 186-191.
- 485 Jia, W., Karaka, K., Parrish, C.R., Naqi, S.A., 1995. A novel variant of infectious bronchitis virus
486 resulting from recombination among three different strains. *Arch Virol*, 140, 259-271.
- 487 Jin, L., Cebra, C.K., Baker, R.J., Mattson, D.E., Cohen, S.A., Alvarado, D.E., Rohrmann, F., 2007.
488 Analysis of the genome sequence of an alpaca coronavirus. *Virology* 365, 198-203.
- 489 Kaneshima, T., Hohdatsu, T., Satoh, K., Takano, T., Motokawa, K., Koyama, H., 2006. The
490 prevalence of a group 2 coronavirus in dogs in Japan. *J. Vet. Med. Sci.* 68, 21-25.
- 491 Kaneshima, T., Hohdatsu, T., Hagino, R., Hosoya, S., Nojiri, Y., Murata, M., Takano, T., Tanabe,
492 M., Tsunemitsu, H., Koyama, H., 2007. The infectivity and pathogenicity of a group 2
493 bovine coronavirus in pups. *J. Vet. Med. Sci.* 69, 301-303.
- 494 Keck, J.G., Matsushima, G.K., Makino, S., Fleming, J.O., Vannier, D.M., Stohlman, S.A., Lai,
495 M.M.C., 1988. In vivo RNA-RNA recombination of coronavirus in mouse brain. *J. Virol.*
496 62, 1810-1813.

- 497 Keenan, K.P., Jervis, H.R., Marchwicki, R.H., Binn, L.N., 1976. Intestinal infection of neonatal
498 dogs with canine coronavirus 1-71: studies by virologic, histologic, histochemical, and
499 immunofluorescent techniques. *Am. J. Vet. Res.* 37, 247-256.
- 500 Kennedy, M., Boedeker, N., Gibbs, P. Kania, S., 2001. Deletions in the 7a ORF of feline
501 coronavirus associated with an epidemic of feline infectious peritonitis. *Vet. Microbiol.* 81,
502 227-234.
- 503 Kirkegaard, K., Baltimore, D., 1986. The mechanism of RNA recombination in poliovirus. *Cell* 47,
504 433-443.
- 505 Kottier, S.A., Cavanagh, D., Britton, P., 1995. Experimental evidence of recombination in
506 coronavirus infectious bronchitis virus. *Virology* 213, 569-580.
- 507 Kumar, S., Tamura, K., Nei, M., 2004. MEGA3: integrated software for Molecular Evolutionary
508 Genetics Analysis and sequence alignment. *Brief. Bioinform.* 5, 150-163.
- 509 Kuo, L., Godeke, G.J., Raamsman, M.J., Masters, P.S., Rottier, P.J., 2000. Retargeting of
510 coronavirus by substitution of the spike glycoprotein ectodomain: crossing the host cell
511 species barrier. *J. Virol.* 74, 1393-1406.
- 512 Lai, M.M.C., Baric, R.S., Makino, S., Keck, J.G., Egbert, J., Leibowitz, J.L., Stohlman, S.A., 1985.
513 Recombination between nonsegmented RNA genomes of murine coronaviruses. *J. Virol.* 56,
514 449-456.
- 515 Lai, M.M.C., Holmes, K.V., 2001. Coronaviridae: The viruses and their replication. In: Knipe,
516 D.M., Howley, P.M. (Eds.), *Fields Virology*, 4th edition. Lippincott Williams and Wilkins,
517 Philadelphia, PA, pp.1163-85.
- 518 Laude, H., Van Reeth, K., Pensaert, M., 1993. Porcine respiratory coronavirus: molecular features
519 and virus-host interactions. *Vet. Res.* 24, 125-150.
- 520 Lee, H.J., Shieh, C.K., Gorbalenya, A.E., Koonin, E.V., La Monica, N., Tuler, J., Bagdzhadzhyan,
521 A., Lai, M.M., 1991. The complete sequence (22 kilobases) of murine coronavirus gene 1
522 encoding the putative proteases and RNA polymerase. *Virology* 180, 567-582.
- 523 Lorusso, A., Haijema, B.J., Decaro, N., Schellen, P., Buonavoglia, C., de Groot, R.J., 2007.
524 Identification and biochemical characterisation of a novel protein unique to canine
525 coronavirus type I, in: *Proceedings of the Third European Congress of Virology*
526 "EuroVirology 2007", Nuernberg, 1-5 September 2007, p..
- 527 Ma, G.G., Lu, C.P., 2005. Two genotypes of Canine coronavirus simultaneously detected in the
528 fecal samples of healthy foxes and raccoon dogs. *Wei Sheng Wu Xue Bao* 45, 305-308.
- 529 Makino, S., Keck, J.G., Stohlman, S.A., Lai, M.M.C., 1986. High-frequency RNA recombination of
530 murine coronavirus. *J. Virol.* 57, 729-739.

- 531 Möstl, K., Buxbaum, A., Odörfer, G., 1994. Verbreitung und Bedeutung von
532 Coronavirusinfektionen in heimischen Hundepopulationen. Wiener Tierärztliche
533 Monatsschrift 84, 355-361.
- 534 Naylor, M.J., Harrison, G.A., Monckton, R.P., McOrist, S., Lehrbach, P.R. and Deane, E.M.,
535 2001a. Identification of canine coronavirus strains from faeces by S gene nested PCR and
536 molecular characterization of a new Australian isolate. *J. Clin. Microbiol.* 39, 1036-1041.
- 537 Naylor, M.J., Monckton, R.P., Lehrbach, P.R., Deane, E.M., 2001b. Canine coronavirus in
538 Australian dogs. *Aust. Vet. J.* 79, 116-119.
- 539 Naylor, M.J., Walia, C.S., McOrist, S., Lehrbach, P.R., Deane, E.M., Harrison, G.A., 2002.
540 Molecular characterization confirms the presence of a divergent strain of canine coronavirus
541 (UWSMN-1) in Australia. *J. Clin. Microbiol.* 40, 3518-3522.
- 542 Pratelli, A., Martella, V., Elia, G., Decaro, N., Aliberti, A., Buonavoglia, D., Tempesta, M.,
543 Buonavoglia, C., 2001. Variation of the sequence in the gene encoding for transmembrane
544 protein M of canine coronavirus (CCV). *Mol. Cell Probes* 15, 229-233.
- 545 Pratelli, A., Elia, G., Martella, V., Tinelli, A., Decaro, N., Marsilio, F., Buonavoglia, D., Tempesta,
546 M. and Buonavoglia, C., 2002. M gene evolution of canine coronavirus in naturally infected
547 dogs. *Vet. Rec.* 151, 758-761.
- 548 Pratelli, A., Martella, V., Decaro, N., Tinelli, A., Camero, M., Cirone, F., Elia, G., Cavalli, A.,
549 Corrente, M., Greco, G., Buonavoglia, D., Gentile, M., Tempesta, M., Buonavoglia, C.,
550 2003a. Genetic diversity of a canine coronavirus detected in pups with diarrhoea in Italy. *J.*
551 *Virol. Methods* 110, 9-17.
- 552 Pratelli, A., Martella, V., Pistello, M., Elia, G., Decaro, N., Buonavoglia, D., Camero, M.,
553 Tempesta, M., Buonavoglia, C., 2003b. Identification of coronaviruses in dogs that
554 segregate separately from the canine coronavirus genotype. *J. Virol. Methods* 107, 213-222.
- 555 Pratelli, A., Decaro, N., Tinelli, A., Martella, V., Elia, G., Tempesta, M., Cirone, F., Buonavoglia,
556 C., 2004a. Two genotypes of canine coronavirus simultaneously detected in fecal samples of
557 dogs with diarrhea. *J. Clin. Microbiol.* 42, 1797-1799.
- 558 Pratelli, A., Elia, G., Decaro, N., Tola, S., Tinelli, A., Martella, V., Rocca, S., Tempesta, M.,
559 Buonavoglia, C., 2004b. Cloning and expression of two fragments of the S gene of canine
560 coronavirus type I. *J. Virol. Methods* 117, 61-65.
- 561 Pratelli, A., Tinelli, A., Decaro, N., Martella, V., Camero, M., Tempesta, M., Martini, M.,
562 Carmichael, L.E., Buonavoglia, C., 2004c. Safety and efficacy of a modified-live canine
563 coronavirus vaccine in dogs. *Vet. Microbiol.* 99, 43-49.

- 564 Priestnall, S.L., Brownlie, J., Dubovi, E.J., Erles, K., 2006. Serological prevalence of canine
565 respiratory coronavirus. *Vet. Microbiol.* 115, 43-53.
- 566 Rimmelzwaan, G.F., Groen, J., Egberink, H., Borst, G.H., UytdeHaag, F.G., Osterhaus, A.D., 1991.
567 The use of enzyme-linked immunosorbent assay systems for serology and antigen detection
568 in parvovirus, coronavirus and rotavirus infections in dogs in The Netherlands. *Vet.*
569 *Microbiol.* 26, 25-40.
- 570 Rottier, P.J.M., 1995. The coronavirus membrane protein. In: Siddell, S.G. (Ed.), *The*
571 *Coronaviridae*. Plenum Press, New York, pp. 115-139.
- 572 Rottier, P.J., Nakamura, K., Schellen, P., Volders, H., Haijema, B.J., 2005. Acquisition of
573 macrophage tropism during the pathogenesis of feline infectious peritonitis is determined by
574 mutations in the feline coronavirus spike protein. *J. Virol.* 79, 14122-14130.
- 575 Sanchez, C.M., Izeta, A., Sanchez-Morgado, J.M., Alonso, S., Sola, I., Balasch, M., Plana-Duran J.,
576 Enjuanes, L., 1999. Targeted recombination demonstrates that the spike gene of
577 transmissible gastroenteritis coronavirus is a determinant of its enteric tropism and
578 virulence. *J. Virol.* 73, 7607-7618.
- 579 Sanchez-Morgado, J.M., Poynter, S., Morris, T.H., 2004. Molecular characterization of a virulent
580 canine coronavirus BGF strain. *Virus Res.* 104, 27-31.
- 581 Schulz, B.S., Strauch, C., Mueller, R.S., Eichhorn, W., Hartmann, K., 2008. Comparison of the
582 prevalence of enteric viruses in healthy dogs and those with acute haemorrhagic diarrhoea
583 by electron microscopy. *J. Small Anim. Pract.* 2007 Dec 14; [Epub ahead of print].
- 584 Song, H.D., Tu, C.C., Zhang, G.W., Wang, S.Y., Zheng, K., Lei, L.C., Chen, Q.X., Gao, Y.W.,
585 Zhou, H.Q., Xiang, H., Zheng, H.J., Chern, S.W., Cheng, F., Pan, C.M., Xuan, H., Chen,
586 S.J., Luo, H.M., Zhou, D.H., Liu, Y.F., He, J.F., Qin, P.Z., Li, L.H., Ren, Y.Q., Liang, W.J.,
587 Yu, Y.D., Anderson, L., Wang, M., Xu, R.H., Wu X.W., Zheng, H.Y., Chen, J.D., Liang,
588 G., Gao, Y., Liao, M., Fang, L., Jiang, L.Y., Li, H., Chen, F., Di, B., He, L.J., Lin, J.Y.,
589 Tong, S., Kong, X., Du, L., Hao, P., Tang, H., Bernini, A., Yu, H.J., Spiga, O., Guo, Z.M.,
590 Pan, H.Y., He, W.Z., Manuguerra, J.C., Fontanet, A., Danchin, A., Niccolai, N., Li, Y.X.,
591 Wu, C.I., Zhao, G.P., 2005. Cross-host evolution of severe acute respiratory syndrome
592 coronavirus in palm civet and human. *Proc. Natl. Acad. Sci. U S A.* 102, 2430-2435.
- 593 Tang, X.C., Zhang, J.X., Zhang, S.Y., Wang, P., Fan, X.H., Li, L.F., Li, G., Dong, B.Q., Liu, W.,
594 Cheung, C.L., Xu, K.M., Song, W.J., Vijaykrishna, D., Poon, L.L., Peiris, J.S., Smith, G.J.,
595 Chen, H., Guan, Y., 2006. Prevalence and genetic diversity of coronaviruses in bats from
596 China. *J. Virol.* 80, 7481-7490.

- 597 Tennant, B.J., Gaskell, R.M., Kelly, D.F., Carter, S.D., Gaskell, C.J., 1991. Canine coronavirus
598 infection in the dog following oronasal inoculation. *Res. Vet. Sci.* 51, 11-18.
- 599 Tennant, B.J., Gaskell, R.M., Jones, R.C., Gaskell, C.J., 1993. Studies on the epizootiology of
600 canine coronavirus. *Vet. Rec.* 132, 7-11.
- 601 Tsunemitsu, H., el-Kanawati, Z.R., Smith, D.R., Reed, H.H., Saif, L.J., 1995. Isolation of
602 coronaviruses antigenically indistinguishable from bovine coronavirus from wild ruminants
603 with diarrhea. *J. Clin. Microbiol.* 33, 3264-3269.
- 604 Vaughn, E.M., Halbur, P.G., Paul, P.S., 1995. Sequence comparison of porcine respiratory
605 coronavirus isolates reveals heterogeneity in the S, 3, and 3-1 genes. *J. Virol.* 69, 3176-3184.
- 606 Vennema, H., Poland, A., Floyd Hawkins, K., Pedersen, N.C., 1995. A comparison of the genomes
607 of FECVs and FIPVs and what they tell us about the relationships between feline
608 coronaviruses and their evolution. *Feline Pract.* 23, 40-44.
- 609 Vennema, H., Godeke, G.J., Rossen, J.W., Voorhout, W.F., Horzinek, M.C., Opstelten, D.J.,
610 Rottier, P.J., 1996. Nucleocapsid-independent assembly of coronavirus-like particles by co-
611 expression of viral envelope protein genes. *EMBO J.* 15, 2020-2028.
- 612 Vennema, H., Poland, A., Foley, J., Pedersen, N.C., 1998. Feline infectious peritonitis viruses arise
613 by mutation from endemic feline enteric coronaviruses. *Virology* 243, 150-157.
- 614 Verbeek, A., Tijssen, P., 1991. Sequence analysis of the turkey enteric coronavirus nucleocapsid
615 and membrane protein genes: a close genomic relationship with bovine coronavirus. *J. Gen.*
616 *Virol.* 72, 1659-1666.
- 617 Vijgen, L., Keyaerts, E., Moes, E., Thoelen, I., Wollants, E., Lemey, P., Vandamme A.M., Van
618 Ranst, M., 2005. Complete genomic sequence of human coronavirus OC43: molecular clock
619 analysis suggests a relatively recent zoonotic coronavirus transmission event. *J. Virol.* 79,
620 1595-1604.
- 621 Vijgen, L., Keyaerts, E., Lemey, P., Maes, P., Van Reeth, K., Nauwynck, H., Pensaert, M., Van
622 Ranst, M., 2006. Evolutionary history of the closely related group 2 coronaviruses: porcine
623 hemagglutinating encephalomyelitis virus, bovine coronavirus, and human coronavirus
624 OC43. *J. Virol.* 80, 7270-7274.
- 625 Wang, Y., Ma, G., Lu, C., Wen, H., 2006. Detection of canine coronaviruses genotype I and II in
626 raised *Canidae* animals in China. *Berl. Munch Tierarztl. Wochenschr* 119, 35-39.
- 627 Weiss, S.R., Navas-Martin, S., 2005. Coronavirus pathogenesis and the emerging pathogen severe
628 acute respiratory syndrome coronavirus. *Microbiol. Mol. Biol. Rev.* 69, 635-64.

- 629 Wesley, R.D., 1999. The S gene of canine coronavirus, strain UCD-1, is more closely related to the
630 S gene of transmissible gastroenteritis virus than to that of feline infectious peritonitis virus.
631 *Virus Res.* 61, 145-152.
- 632 Wesseling, J.G., Vennema, H., Godeke, G.J., Horzinek, M.C., Rottier, P.J., 1994. Nucleotide
633 sequence and expression of the spike (S) gene of canine coronavirus and comparison with
634 the S proteins of feline and porcine coronaviruses. *J. Gen. Virol.* 75, 1789-1794.
- 635 Wise, A.G., Kiupel, M., Maes, R.K., 2006. Molecular characterization of a novel coronavirus
636 associated with epizootic catarrhal enteritis (ECE) in ferrets. *Virology* 349, 164-174.
- 637 Woo, P.C., Lau, S.K., Chu, C.M., Chan, K.H., Tsoi, H.W., Huang, Y., Wong, B.H., Poon, R.W.,
638 Cai, J.J., Luk, W.K., Poon, L.L., Wong, S.S., Guan, Y., Peiris, J.S., Yuen, K.Y., 2005.
639 Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1,
640 from patients with pneumonia. *J. Virol.* 79, 884-895.
- 641 Yamanaka, M., Crisp, T., Brown, R., Dale, B., 1998. Nucleotide sequence of the inter-structural
642 gene region of feline infectious peritonitis virus. *Virus Genes* 16, 317-318.
- 643 Yeşilbağ, K., Yılmaz, Z., Torun, S., Pratelli, A., 2004. Canine coronavirus infection in Turkish dog
644 population. *J. Vet. Med. B Infect. Dis. Vet. Public Health* 51, 353-355.
- 645 Zhang, X.M., Herbst, W., Kousoulas, K.G., Storz, J., 1994. Biological and genetic characterization
646 of a hemagglutinating coronavirus isolated from a diarrhoeic child. *J. Med. Virol.* 44, 152-
647 161.

648 **Figure legends.**

649 **Fig. 1.** Phylogenetic relationship of the S proteins of animal and human coronaviruses. The tree was
650 generated by the neighbor-joining method in the Mega3 program (Kumar et al., 2004). For
651 phylogenetic tree construction, the following CoV strains were used (GenBank accession numbers
652 are reported in parentheses): group 1: canine coronavirus type II (CCoVII) Insavc1 (D13096),
653 CCoVII-BGF10 (AY342160), CCoVII-CB/05 (DQ112226), canine coronavirus type I (CCoVI)
654 Elmo/02 (AY307020), CCoV-I-23/03 (AY307021), feline coronavirus type II (FCoVII) 79-1146
655 (NC_007025), FCoV-II-79-1683 (X80799), feline coronavirus type I (FCoVI) KU-2 (D32044),
656 FCoV-I-Black (AB088223), FCoV-I-UCD1 (AB088222), transmissible gastroenteritis virus (TGEV)
657 Purdue (NC_002306), Chinese ferret badger coronavirus (CFBCoV) CFB/GD/DM95/03
658 (EF192156), human coronavirus (HCoV) NL63 (NC_005831); group 2: human severe acute
659 respiratory syndrome coronavirus (SARS-CoV) Tor2 (NC_004718), BCoV-Mebus (U00735),
660 giraffe coronavirus (GiCoV) US/OH3/2003 (EF424623), alpaca coronavirus (ACoV) (DQ915164),
661 sable antelope coronavirus (SACoV) US/OH1/2003 (EF424621), bubaline coronavirus (BuCoV)
662 179/07-11 (EU019216), canine respiratory coronavirus (CRCoV) 4182 (DQ682406), HCoV-OC43
663 ATCC VR-759 (NC_005147), human enteric coronavirus (HECoV) 4408 (L07748), porcine
664 haemagglutinating encephalomyelitis virus (PHEV) VW572 (DQ011855), mouse hepatitis virus
665 (MHV) A59 (AY700211), rat sialodacryoadenitis virus (SDAV) 681 (AF207551), HCoV-HKU1
666 (NC_006577), equine coronavirus (ECoV) NC99 (NC_010327); group 3: avian infectious bronchitis
667 virus (IBV) Beaudette (NP_040831); turkey coronavirus (TCoV) G1 (AY342357). Coronaviruses of
668 dogs are grey shaded. A statistical support was provided by bootstrapping over 1,000 replicates.
669 The scale bar indicates the estimated numbers of amino acid substitutions per site.

670

671 **Fig. 2.** Schematic representation of the genomes of CCoVs and FCoVs depicting the genetic
672 differences among the CCoV genotypes/biotypes. Genes encoding for structural and non-structural
673 proteins are shown in grey and white, respectively. ORF sizes are not drawn to scale. The arrows

674 indicate the transcription regulating sequences preceding each CoV gene. The length in amino acids
675 of the nsp 3b of strains BGF10 and CB/05 and the 38-nt deletion in ORF3b of strain CB/05 are
676 reported.

677

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Fig. 1

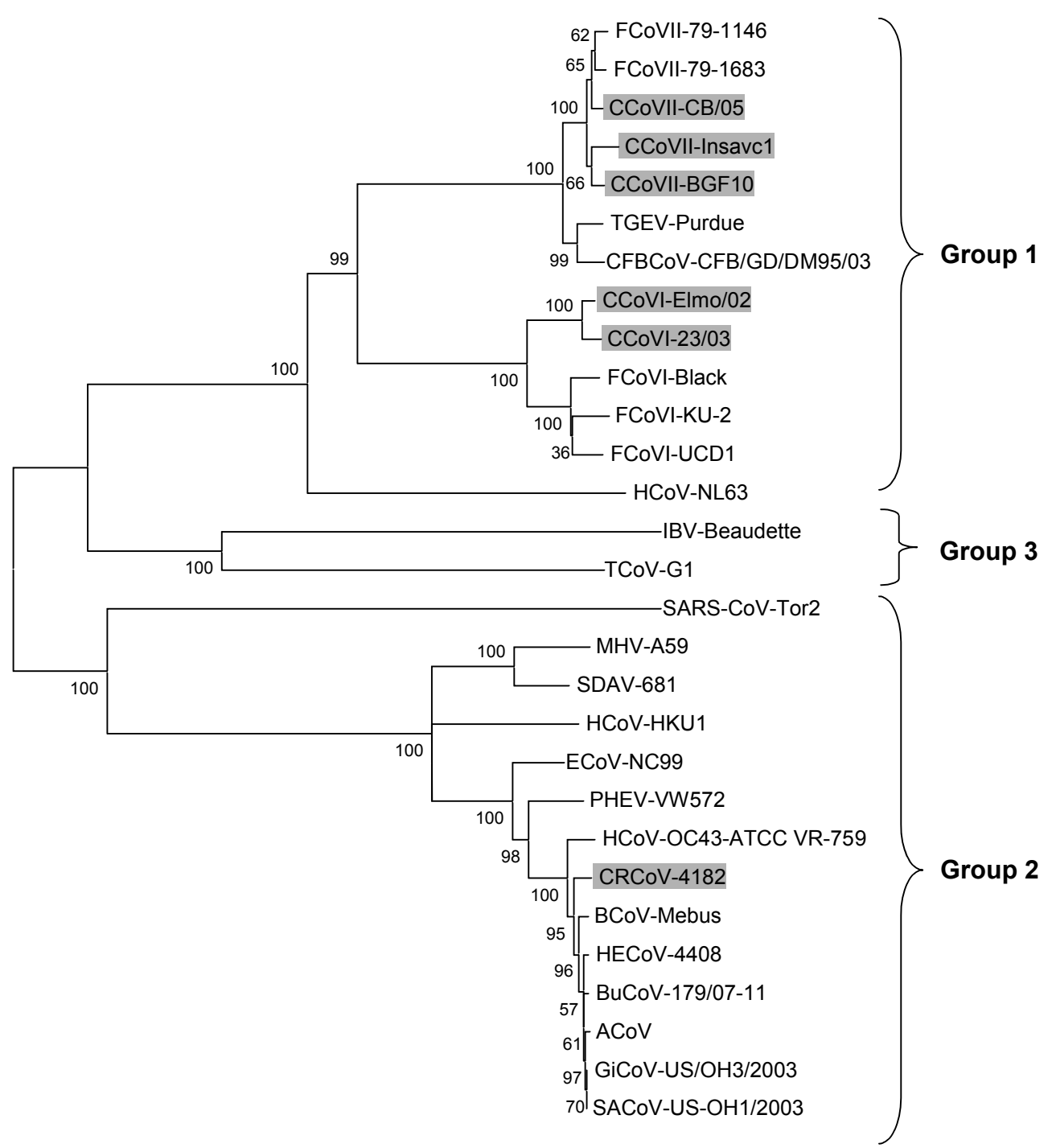


Fig. 2

