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An update on canine coronaviruses: viral evolution and pathobiology

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Abstract

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

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

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Coronavirus taxonomy and genomic organisation

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

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

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

Canine enteric coronavirus (CCoV)

History and pathobiology

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

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

CCoV genotypes

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

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

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

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

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

Virulent/divergent strains

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

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

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

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

Canine pantropic coronavirus

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

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

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

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

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

Canine respiratory coronavirus (CRCoV)

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

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

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

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

Epilogue

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

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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

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

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

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

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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.

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References

Bandai, C., Ishiguro, S., Masuya, N., Hohdatsu, T. and Mochizuki, M., 1999. Canine coronavirus infections in Japan: virological and epidemiological aspects. J. Vet. Med. Sci. 61, 731-736.

Benetka, V., Kolodziejek, J., Walk, K., Rennhofer, M., Möstl, K., 2006. M gene analysis of atypical strains of feline and canine coronavirus circulating in an Austrian animal shelter. Vet. Rec. 159, 170-174.

Binn, L.N., Lazar, E.C., Keenan, K.P., Huxsoll, D.L., Marchwicki, R.H., Strano, A.J., 1974. Recovery and characterization of a coronavirus from military dogs with diarrhea. Proc. Annu. Meet. U. S. Anim. Health Assoc. 78, 359-366.

Boursnell, M.E., Brown, T.D., Foulds, I.J., Green, P.F., Tomley, F.M., Binns, M.M., 1987. Completion of the sequence of the genome of the coronavirus avian infectious bronchitis virus.

J. Gen. Virol. 68, 57-77.

Buonavoglia, C., Decaro, N., Martella, V., Elia, G., Campolo, M., Desario, C., Castagnaro, M., Tempesta, M., 2006. Canine coronavirus highly pathogenic for dogs. Emerg. Infect. Dis. 12, 492-494.

Buonavoglia, C., Martella, V., 2007. Canine respiratory viruses. Vet. Res. 38, 355-73.

Carmichael, L.E., 1978. Infectious canine enteritis caused by corona-like virus: current status and request for information. Baker Institute Laboratory Report, series 2, no. 9. James A. Baker Insitute for Animal Health, Ithaca, N.Y.

Collins, A.R., Knobler, R.L., Powell, H., Buchmeier, M.J., 1982. Monoclonal antibodies to murine hepatitis virus-4 (strain JHM) define the viral glycoprotein responsible for attachment and cell-cell fusion. Virology 119, 358-371.

Cooper, P.D., Steiner-Pryor, S., Scotti, P.D., Delong, D., 1974. On the nature of poliovirus genetic recombinants. J. Gen. Virol. 23, 41-49.

Dea, S., Verbeek, A.J., Tijssen, P., 1990. Antigenic and genomic relationships among turkey and bovine enteric coronaviruses. J. Virol. 64, 3112-3118.

Decaro, N., Camero, M., Greco, G., Zizzo, N., Elia, G., Campolo, M., Pratelli, A., Buonavoglia, C., 2004a. Canine distemper and related diseases: report of a severe outbreak in a kennel. New Microbiol. 27, 177-181.

Decaro, N., Pratelli, A., Campolo, M., Elia, G., Martella, V., Tempesta, M., Buonavoglia, C., 2004b. Quantitation of canine coronavirus RNA in the faeces of dogs by TaqMan RT-PCR. J. Virol. Methods 119, 145-150.

Accepted Manuscript

ACCEPTED MANUSCRIPT

Decaro, N., Martella, V., Ricci, D., Elia G, Desario C, Campolo M, Cavaliere N, Di Trani L, Tempesta M, Buonavoglia C., 2005. Genotype-specific fluorogenic RT-PCR assays for the detection and quantitation of canine coronavirus type I and type II RNA in faecal samples of dogs. J. Virol. Methods 130, 72-78.

Decaro, N., Martella, V., Desario, C., Bellacicco, A.L., Camero, M., Manna, L., D'aloja, D., Buonavoglia, C., 2006. First detection of canine parvovirus type 2c in pups with haemorrhagic enteritis in Spain. J. Vet. Med. B Infect. Dis. Vet. Public Health 53, 468-472.

Decaro, N., Campolo, M., Elia, G., Buonavoglia, D., Colaianni, M.L., Lorusso, A., Mari, V., Buonavoglia C., 2007a. Infectious canine hepatitis: An "old" disease reemerging in Italy. Res. Vet. Sci. 83, 269-273.

Decaro, N., Desario, C., Elia, G., Campolo, M., Lorusso, A., Mari, V., Martella, V., Buonavoglia, C., 2007b. Occurrence of severe gastroenteritis in pups after canine parvovirus vaccine administration: a clinical and laboratory diagnostic dilemma. Vaccine 25, 1161-1166.

Decaro, N, Desario, C., Elia, G., Mari, V., Lucente, M.S., Cordioli, P., Colaianni, M.L., Martella, V., Buonavoglia C., 2007c. Serological and molecular evidence that canine respiratory coronavirus is circulating in Italy. Vet. Microbiol. 121, 225-230.

Decaro, N., Martella, V., Elia, G., Campolo, M., Desario, C., Cirone, F., Tempesta, M., Buonavoglia, C., 2007d. Molecular characterisation of the virulent canine coronavirus CB/05 strain. Virus Res., 125, 54-60.

Decaro, N., Campolo, M., Lorusso, A., Desario, C., Mari, V., Colaianni, M.L., Elia, G., Martella, V., Buonavoglia, C., 2008a. Experimental infection of dogs with a novel strain of canine coronavirus causing systemic disease and lymphopenia. Vet. Microbiol. 128, 253-260.

Decaro, N., Martella, V., Elia, G., Campolo, M., Mari, V., Desario, C., Lucente, M.S., Lorusso, A., Greco, G., Corrente, M., Tempesta, M., Buonavoglia, C., 2008b. Biological and genetic analysis of a bovine-like coronavirus isolated from water buffalo (*Bubalus bubalis*) calves. Virology 370, 213-222.

Dolja, V.V., Carrington, J.C., 1992. Evolution of positive-strand RNA viruses. Semin. Virol. 3, 315-326.

Dong, B.Q., Liu, W., Fan, X.H., Vijaykrishna, D., Tang, X.C., Gao, F., Li, L.F., Li, G.J., Zhang, J.X., Yang, L.Q., Poon, L.L., Zhang, S.Y., Peiris, J.S., Smith, G.J., Chen, H., Guan, Y., 2007. Detection of a novel and highly divergent coronavirus from asian leopard cats and Chinese ferret badgers in Southern China. J. Virol. 81, 6920-6926.

Eleouet, J.F., Rasschaert, D., Lambert, P., Levy, L., Vende, P., Laude, H., 1995. Complete genomic sequence of the transmissible gastroenteritis virus. Adv. Exp. Med. Biol. 380, 459-461.

ACCEPTED MANUSCRIPT

Ellis, J.A., McLean, N., Hupaelo, R., Haines, D.M., 2005. Detection of coronavirus in cases of tracheobronchitis in dogs: a retrospective study from 1971 to 2003. Can. Vet. J. 46, 447-448.

Enjuanes, L., Brian, D., Cavanagh, D., Holmes, K., Lai, M.M.C., Laude, H., Masters, P., Rottier, P., Siddell, S., Spaan, W.J.M., Taguchi, F., Talbot, P., 2000. Family Coronaviridae, in: van Regenmortel, M.H.V., Fauquet, C.M., Bishop, D.H.L., Carstens, E.B., Estes, M.K., Lemon, S.M., Maniloff, J., Mayo, M.A., McGeoch, D.J., Pringle, C.R., Wickner, R.B. (Eds.), Virus Taxonomy, Classification and Nomenclature of Viruses. Academic Press, New York, pp. 835-849.

Erles, K., Toomey, C., Brooks, H.W., Brownlie, J., 2003. Detection of a group 2 coronavirus in dogs with canine infectious respiratory disease. Virology 310, 216-223.

Erles, K., Dubovi, E.J., Brooks, H.W., Brownlie, J., 2004. Longitudinal study of viruses associated with canine infectious respiratory disease. J. Clin. Microbiol. 42, :4524-4529.

Erles, K., Brownlie, J., 2005. Investigation into the causes of canine infectious respiratory disease: antibody responses to canine respiratory coronavirus and canine herpesvirus in two kennelled dog populations. Arch. Virol. 150, 1493-1504.

Erles, K., Kai-Biu, S., Brownlie, J., 2006. Isolation and sequence analysis of canine respiratory coronavirus. Virus Res. 124, 78-87.

Escutenaire, S., Isaksson, M., Renström, L.H., Klingeborn, B., Buonavoglia, C., Berg, M., Belák, S., Thorén, P., 2007. Characterization of divergent and atypical canine coronaviruses from Sweden.

Arch. Virol. 152, 1507-1514.

Evermann, J.F., Abbott, J.R., Han, S., 2005. Canine coronavirus-associated puppy mortality without evidence of concurrent canine parvovirus infection. J. Vet. Diagn. Invest. 17, 610-614.

Gebauer, F., Posthumus, W.A.P., Correa, I., Suñé, C., Sánchez, C.M., Smerdou, C., Lenstra, J.A., Meloen, R., Enjuanes, L., 1991. Residues involved in the formation of the antigenic sites of the S protein of transmissible gastroenteritis coronavirus. Virology 183, 225-238.

Gorbalenya, A.E., Snijder, E.J., Spaan, W.J., 2004. Severe acute respiratory syndrome coronavirus phylogeny: toward consensus. J. Virol. 78, 7863-7866.

Guan, Y., Zheng, B.J., He, Y.Q., Liu, X.L., Zhuang, Z.X., Cheung, C.L., Luo, S.W., Li, P.H., Zhang, L.J., Guan, Y.J., Butt, K.M., Wong, K.L., Chan, K.W., Lim, W., Shortridge, K.F., Yuen, K.Y., Peiris, J.S., Poon, L.L., 2003. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science 302, 276-278.

Guy, J.S., Breslin, J.J., Breuhaus, B., Vivrette, S., Smith, LG., 2000. Characterization of a coronavirus isolated from a diarrheic foal. J. Clin. Microbiol. 38, 4523-4526.

ACCEPTED MANUSCRIPT

Haijema, B.J., Volders, H., Rottier, P.J., 2003. Switching species tropism: an effective way to manipulate the feline coronavirus genome. J. Virol. 77, 4528-4538.

Haijema, B.J., Volders, H., Rottier, P.J., 2004. Live, attenuated coronavirus vaccines through the directed deletion of group-specific genes provide protection against feline infectious peritonitis. J. Virol. 78, 3863-3871.

Hasoksuz, M, Alekseev, K., Vlasova, A., Zhang, X., Spiro, D., Halpin, R., Wang, S., Ghedin, E., Saif, L.J., 2007. Biologic, antigenic, and full-length genomic characterization of a bovine-like coronavirus isolated from a giraffe. J. Virol. 81, 4981-4990.

Haspel, M.V., Lampert, P.W., Oldstone, M.B., 1978. Temperature-sensitive mutants of mouse hepatitis virus produce a high incidence of demyelination. Proc. Natl. Acad. Sci. U.S.A. 75, 4033-4036.

Herold, J., Raabe, T., Siddell, S., 1993. Molecular analysis of the human coronavirus (strain 229E) genome. Arch. Virol. Suppl. 7, 63-74.

Herrewegh, A.A.P.M., Smeenk, I., Horzinek, M.C., Rottier, P.J.M., de Groot, R.J., 1998. Feline coronavirus type II strains 79-1683 and 79-1146 originate from a double recombination between feline coronavirus type I and canine coronavirus. J. Virol. 72, 4508-4514.

Horsburgh, B.C., Brown, T.D., 1995. Cloning, sequencing and expression of the S protein gene from two geographically distinct strains of canine coronavirus. Virus Res. 39, 63-74.

Jarvis, T.C., Kirkegaard, K., 1991. The polymerase in its labyrinth: mechanisms and implications of RNA recombination. Trends Genet. 7, 186-191.

Jia, W., Karaka, K., Parrish, C.R., Naqi, S.A., 1995. A novel variant of infectious bronchitis virus resulting from recombination among three different strains. Arch Virol, 140, 259-271.

Jin, L., Cebra, C.K. , Baker, R.J., Mattson, D.E., Cohen, S.A., Alvarado, D.E., Rohrmann, F., 2007. Analysis of the genome sequence of an alpaca coronavirus. Virology 365, 198-203.

Kaneshima, T., Hohdatsu, T., Satoh, K., Takano, T., Motokawa, K., Koyama, H., 2006. The prevalence of a group 2 coronavirus in dogs in Japan. J. Vet. Med. Sci. 68, 21-25.

Kaneshima, T., Hohdatsu, T., Hagino, R., Hosoya, S., Nojiri, Y., Murata, M., Takano, T., Tanabe, M., Tsunemitsu, H., Koyama, H., 2007. The infectivity and pathogenicity of a group 2 bovine coronavirus in pups. J. Vet. Med. Sci. 69, 301-303.

Keck, J.G., Matsushima, G.K., Makino, S., Fleming, J.O., Vannier, D.M., Stohlman, S.A., Lai, M.M.C., 1988. In vivo RNA-RNA recombination of coronavirus in mouse brain. J. Virol. 62, 1810-1813.

Accepted Manuscript

ACCEPTED MANUSCRIPT

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.

ACCEPTED MANUSCRIPT

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 oronavirus 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.

Accepted Manuscript

ACCEPTED MANUSCRIPT

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.

Accepted Manuscript

ACCEPTED MANUSCRIPT

Tennant, B.J., Gaskell, R.M., Kelly, D.F., Carter, S.D., Gaskell, C.J., 1991. Canine coronavirus infection in the dog following oronasal inoculation. Res. Vet. Sci. 51, 11-18.

Tennant, B.J., Gaskell, R.M., Jones, R.C., Gaskell, C.J., 1993. Studies on the epizootiology of canine coronavirus. Vet. Rec. 132, 7-11.

Tsunemitsu, H,. el-Kanawati, Z.R , Smith, D.R., Reed, H.H., Saif, L.J., 1995. Isolation of coronaviruses antigenically indistinguishable from bovine coronavirus from wild ruminants with diarrhea. J. Clin. Microbiol. 33, 3264-3269.

Vaughn, E.M., Halbur, P.G., Paul, P.S., 1995. Sequence comparison of porcine respiratory coronavirus isolates reveals heterogeneity in the S, 3, and 3-1 genes. J. Virol. 69, 3176-3184.

Vennema, H., Poland, A., Floyd Hawkins, K., Pedersen, N.C., 1995. A comparison of the genomes of FECVs and FIPVs and what they tell us about the relationships between feline coronaviruses and their evolution. Feline Pract. 23, 40-44.

Vennema, H., Godeke, G.J., Rossen, J.W., Voorhout, W.F., Horzinek, M.C., Opstelten, D.J., Rottier, P.J., 1996. Nucleocapsid-independent assembly of coronavirus-like particles by co-expression of viral envelope protein genes. EMBO J. 15, 2020-2028.

Vennema, H., Poland, A., Foley, J., Pedersen, N.C., 1998. Feline infectious peritonitis viruses arise by mutation from endemic feline enteric coronaviruses. Virology 243, 150–157.

Verbeek, A., Tijssen, P., 1991. Sequence analysis of the turkey enteric coronavirus nucleocapsid and membrane protein genes: a close genomic relationship with bovine coronavirus. J. Gen. Virol. 72, 1659-1666.

Vijgen, L, Keyaerts, E., Moes, E., Thoelen, I., Wollants, E., Lemey, P., Vandamme A.M., Van Ranst, M., 2005. Complete genomic sequence of human coronavirus OC43: molecular clock analysis suggests a relatively recent zoonotic coronavirus transmission event. J. Virol. 79, 1595-1604.

Vijgen, L, Keyaerts, E., Lemey, P., Maes, P., Van Reeth, K., Nauwynck, H., Pensaert, M., Van Ranst, M., 2006. Evolutionary history of the closely related group 2 coronaviruses: porcine hemagglutinating encephalomyelitis virus, bovine coronavirus, and human coronavirus OC43. J. Virol. 80, 7270-7274.

Wang, Y., Ma, G., Lu, C., Wen, H., 2006. Detection of canine coronaviruses genotype I and II in raised *Canidae* animals in China. Berl. Munch Tierarztl. Wochenschr 119, 35-39.

Weiss, S.R., Navas-Martin, S., 2005. Coronavirus pathogenesis and the emerging pathogen severe acute respiratory syndrome coronavirus. Microbiol. Mol. Biol. Rev. 69, 635-64.

Accepted Manuscript

ACCEPTED MANUSCRIPT

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., Yilmaz, 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.

Accepted Manuscript

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Figure legends.

Fig. 1. Phylogenetic relationship of the S proteins of animal and human coronaviruses. The tree was generated by the neighbor-joining method in the Mega3 program (Kumar et al., 2004). For phylogenetic tree construction, the following CoV strains were used (GenBank accession numbers are reported in parentheses): group 1: canine coronavirus type II (CCoVII) Insavc1 (D13096), CCoVII-BGF10 (AY342160), CCoVII-CB/05 (DQ112226), canine coronavirus type I (CCoVI) Elmo/02 (AY307020), CCoVI-23/03 (AY307021), feline coronavirus type II (FCoVII) 79-1146 (NC_007025), FCoVII-79-1683 (X80799), feline coronavirus type I (FCoVI) KU-2 (D32044), FCoVI-Black (AB088223), FCoVI-UCD1 (AB088222), transmissible gastroenteritis virus (TGEV) Purdue (NC_002306), Chinese ferret badger coronavirus (CFBCoV) CFB/GD/DM95/03 (EF192156), human coronavirus (HCoV) NL63 (NC_005831); group 2: human severe acute respiratory syndrome coronavirus (SARS-CoV) Tor2 (NC 004718), BCoV-Mebus (U00735), giraffe coronavirus (GiCoV) US/OH3/2003 (EF424623), alpaca coronavirus (ACoV) (DQ915164), sable antelope coronavirus (SACoV) US/OH1/2003 (EF424621), bubaline coronavirus (BuCoV) 179/07-11 (EU019216), canine respiratory coronavirus (CRCoV) 4182 (DQ682406), HCoV-OC43 ATCC VR-759 (NC_005147), human enteric coronavirus (HECoV) 4408 (L07748), porcine haemagglutinating encephalomyelitis virus (PHEV) VW572 (DQ011855), mouse hepatitis virus (MHV) A59 (AY700211), rat sialodacryoadenitis virus (SDAV) 681 (AF207551), HCoV-HKU1 (NC_006577), equine coronavirus (ECoV) NC99 (NC 010327); group 3: avian infectious bronchitis virus (IBV) Beaudette (NP 040831); turkey coronavirus (TCoV) G1 (AY342357). Coronaviruses of dogs are grey shaded. A statistical support was provided by bootstrapping over 1,000 replicates. The scale bar indicates the estimated numbers of amino acid substitutions per site.

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

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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.
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Fig. 1

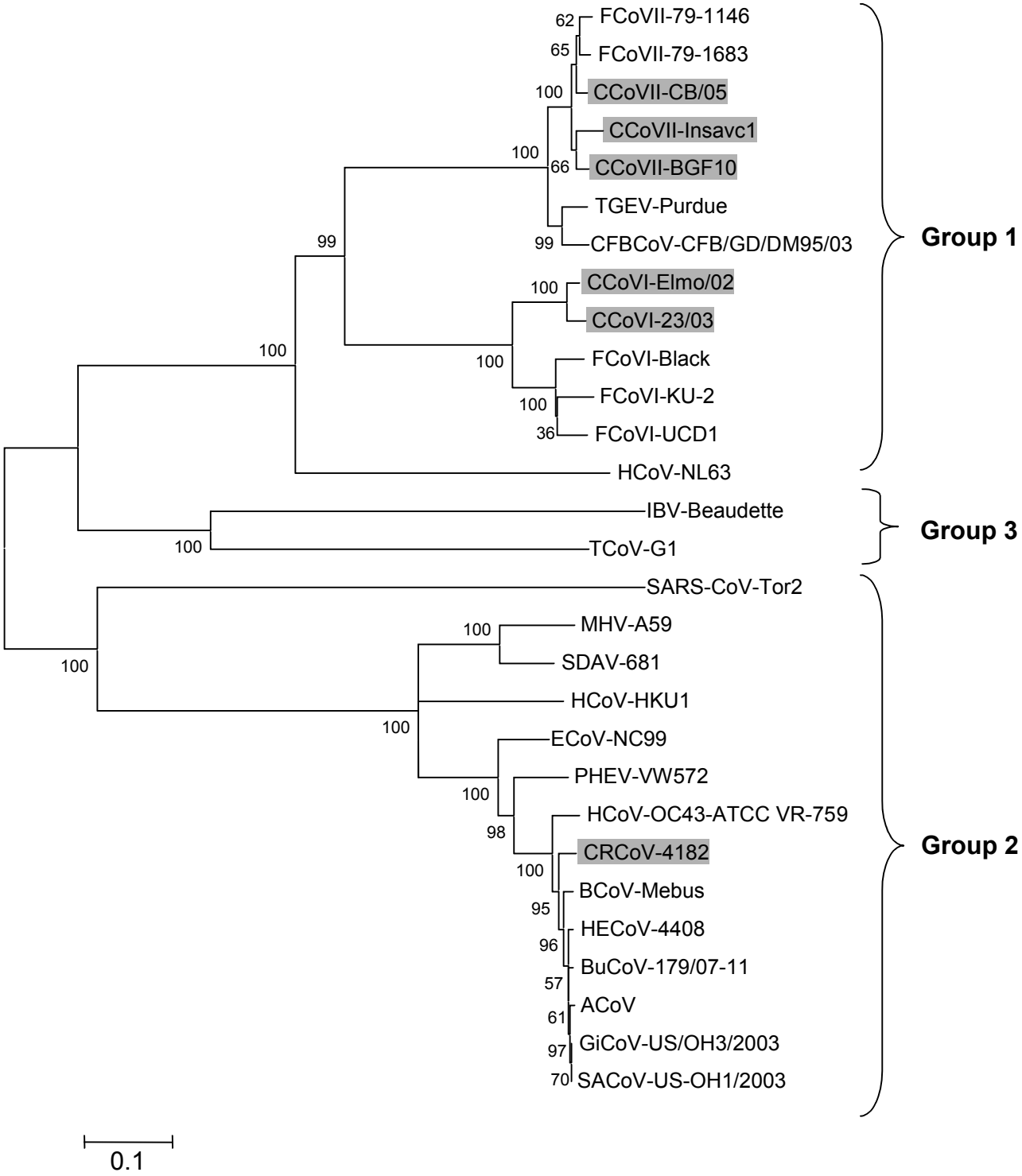

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Fig. 2