Novel human coronavirus (SARS-CoV-2): A lesson from animal coronaviruses

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\textbf{ABSTRACT}

The recent pandemic caused by the novel human coronavirus, referred to as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), not only is having a great impact on the health care systems and economies in all continents but it is also causing radical changes of common habits and life styles. The novel coronavirus (CoV) recognises, with high probability, a zoonotic origin but the role of animals in the SARS-CoV-2 epidemiology is still largely unknown. However, CoVs have been known in animals since several decades, so that veterinary coronavirologists have a great expertise on how to face CoV infections in animals, which could represent a model for SARS-CoV-2 infection in humans.

In the present paper, we provide an up-to-date review of the literature currently available on animal CoVs, focusing on the molecular mechanisms that are responsible for the emergence of novel CoV strains with different antigenic, biologic and/or pathogenetic features. A full comprehension of the mechanisms driving the evolution of animal CoVs will help better understand the emergence, spreading, and evolution of SARS-CoV-2.

1. Introduction

Eighteen years after the emergence of severe acute respiratory syndrome (SARS) in China and 8 years after the emergence of Middle East respiratory syndrome (MERS) in Saudi Arabia, a novel coronavirus (CoV) epidemic, recently classified as pandemic by the WHO, is threatening the human population worldwide (Zhou et al., 2020). The disease, now referred to as coronavirus disease 2019 (COVID-19), is caused by a novel human CoV, which was initially denominated 2019 novel coronavirus (2019-nCoV) and later renamed as SARS coronavirus 2 (SARS-CoV-2) by the Coronavirus Study Group of the International Committee on Taxonomy of Viruses (Gorbalenya et al., 2020). COVID-19 emerged in December 2019 in Wuhan City, Hubei Province, China, in humans exposed to wildlife at the Huanan seafood wholesale market, which is the largest seafood market in central China, and where different species of farm and wild animals are commonly sold (Lorusso et al., 2020). The epidemic has then expanded not only to neighbouring Asian countries, but also to other continents (https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200415-sitrep-86-covid-19.pdf?sfvrsn=c615ea20_2).

A list of human CoVs is showed in Table 1. Historically, only two human CoVs (HCoVs) had been known before the SARS emergence, namely HCoV-229E, an alphacoronavirus originated in bats and transmitted to humans through alpacas, and HCoV-OC43, a betacoronavirus which had passed from rodents to humans through cattle (Corman et al., 2015,2018). After 2002–2003 SARS epidemic, the renewed interest in HCoVs allowed the discovery of two additional viruses, the alphacoronavirus HCoV-NL63 and the betacoronavirus HCoV-HKU1, derived from bats and rodents, respectively (Tao et al., 2017). All these four viruses are usually responsible for mild respiratory symptoms in immunocompetent patients. SARS-CoV and MERS-CoV are two unrelated betacoronaviruses originated in bats and transmitted to humans by wild carnivores and dromedary camels, respectively. In contrast to other HCoVs, these two viruses displayed an increased virulence, causing severe pneumonia and even the death of affected people, with mortality rates of about 10 % and 30 %, respectively (Guarner, 2020).

The occurrence of three highly pathogenic CoVs with a zoonotic origin in less than two decades, highlights the role of animals in generating CoVs with increased virulence that can adapt to humans, causing epidemics (and eventually pandemics) with high impact on human health. Indeed, CoV infections of veterinary interest have been known since almost a century (Cavanagh, 2007; Pedersen, 2014; Decaro et al., 2020), so that animal CoVs are paradigmatic of how this large family of viruses evolves, generating strains with different biological properties. In addition, the efforts done in veterinary medicine...
Table 1

| CoV genus | CoV subgenus | CoV species | CoV common name(s) | Possible ancestor | Associated disease | Reference |
|-----------|--------------|-------------|-------------------|------------------|-------------------|-----------|
| Alphacoronavirus | Setracovirus | Human coronavirus NL63 | HCoV-NL63 | NL63-related bat CoV strain BtKYNL63-9b | Mild respiratory disease | Fouchier et al. (2004) |
| Alphacoronavirus | Duvinacovirus | Human coronavirus 229E | HCoV-229E | Alpaca(alpha)coronavirus ACoV | Mild respiratory disease | Hamre and Procknow |
| Betacoronavirus | Embecovirus | Betacoronavirus 1 | | | | |
| Betacoronavirus | Embecovirus | Human coronavirus HKU1 | HCoV-HKU1 | | A coronavirus strain of Rhinolophus bats. | Woo et al. (2005) |
| Betacoronavirus | Sarbecovirus | Severe acute respiratory syndrome-SARS-CoV | SARS-CoV | Intermediate host: palm masked civets and other wild carnivores | Severe respiratory distress, diarrhoea and vomiting (1/3 patients); 10% case fatality rate | | |
| Betacoronavirus | Sarbecovirus | Severe acute respiratory syndrome-MERS-CoV | MERS-CoV | Intermediate host: dromedary camels | Severe respiratory distress, diarrhoea and vomiting (1/3 patients); 36% case fatality rate | Zhou et al. (2020); Wu et al. (2020) |
| Betacoronavirus | Sarbecovirus | Severe acute respiratory syndrome-SARS-CoV-2 | | | Unknown; 96.2% of nucleotide identity with SARS-rCoV related coronavirus | | | |

The aim of this paper is to present a comprehensive review of the current literature on animal CoVs, their intermingled evolution, characterised by the continuous generation of strains with new pathobiological features and host range.

2. Coronaviruses: changing viruses in a changing world

Coronaviruses (subfamily Orthocoronavirinae, family Coronaviridae, order Nidovirales) are enveloped, single-strand, positive-sense RNA viruses. Currently, four different genera exist, i.e., Alphacoronavirus, Betacoronavirus, Gammacoronavirus and Deltacoronavirus, whose reservoirs are bats and rodents for alpha- and betacoronaviruses or birds for gamma- and deltagammaronaviruses. From their natural reservoirs CoVs may jump to other animals, including humans, with the transmission to humans usually requiring an intermediate host (Lorusso et al., 2020). Each CoV genus is organised in subgenera that are presently 13, 5, 4 and 2 for alpha-, beta-, delta- and gammacoronaviruses, respectively (https://talk.ictvonline.org/taxonomy/).

Among RNA viruses, CoVs possess the largest genome, 27.6–31 kb in size. At the very 5′-end of the genome is a leader sequence, which plays critical roles in the gene expression of CoV during its discontinuous sub-genomic replication (Li et al., 2005). The 5′-most two-thirds of the genome comprises the replicase gene, which consists of two overlapping open reading frames, ORF 1a and 1b. Located downstream of ORF1b are 4 ORFs that code for a common set, to all CoVs, of two overlapping open reading frames, ORF 1a and 1b. Located downstream of ORF1b are 4 ORFs that code for a common set, to all CoVs, of structural proteins (spike (S), envelope (E), membrane (M) and nucleocapsid (N) proteins). The order of the structural protein genes is always conserved in all CoVs. The S protein mediates viral attachment to specific cell receptors and fusion between the envelope and plasma membrane and is the main inducer of virus-neutralising antibodies. The small membrane (E) protein plays an important role in viral envelope assembly, but it is not essential for virus propagation. 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 carboxy-terminal inner domain. The nucleocapsid (N) protein is a highly basic phosphoprotein that in addition to its function in the virion also modulates viral RNA synthesis. In addition to the common set of proteins, CoVs related to bovine coronavirus (BCoV), recently included in the subgenus Embecovirus (genus Betacoronavirus), possess an additional structural protein, the haemagglutinin-esterase (HE), closely related to the haemagglutinin-esterase fusion protein of influenza C virus. CoVs do also possess accessory genes coded by additional ORFs located downstream of ORF1b. Their number, nucleotide sequence and order can vary remarkably among different CoVs. The function of the accessory proteins is in most cases unknown and as a rule they are not essential for virus replication. They do play an important role, however, in virus host interactions as they are generally maintained during natural infection and their loss — either through spontaneous mutation or reversed genetics — results in reduced virulence (Brian and Baric, 2005; Decaro and Buonavoglia, 2008).

Replication of the CoV RNA involves the synthesis of a full-length negative-strand RNA that is present at low concentrations and serves as a template for the synthesis of full-length genomic RNA. The genes downstream of ORF1b are expressed through a 3′-coterminal nested set of subgenomic (sg) mRNAs. According to the generally accepted model for CoV transcription, sg minus-strand RNAs are produced via a discontinuous 3′-extension step, which is regulated by transcription regulating sequences (TRSs) that are present upstream of (most) ORFs and also at the 5′-end of the genome. The minus-strand RNAs in turn serve as templates for the synthesis of complementary sg mRNAs, of which only the 5′ end is generally translated (Brian and Baric, 2005).

CoVs are characterised by an exceptional genetic plasticity and to develop effective vaccines and antiviral therapies against well-known CoV infections of animals could be useful to set up prophylactic and therapeutical strategies against SARS-CoV-2.
emergence of SARS-CoV in 2002, there has been increased interest in far and their associated diseases. The number of avian species in which receptors of new animal species, increasing the viral cause the progressive adaptation of the viral surface proteins to the cell receivers of new animal species, increasing the viral fitness. In addition, the particular replicating machinery of CoVs facilitates recombination events due to the presence of consensus sequences upstream each gene. Therefore, in the case of coinfection by more than one CoV strain, the RNA polymerase can jump from the RNA of a strain to that of the other one, synthetizing a hybrid RNA containing sequences from both viruses. Recombination can occur not only with genomic sequences of other CoVs (homologous recombination), but also with RNAs of different viruses and other organisms (heterologous recombination) (Luytjes et al., 1988; Banner and Lai, 1991; Lai, 1996; Zeng et al., 2008; Huang et al., 2016). Recombination is an alternative mechanism that let CoVs acquire novel biological properties in terms of virulence, host range and tissue tropism, so that CoV strains, which are non-pathogenic or low-pathogenic in the original host, may increase their pathogenicity in the same species or adapt to different species spreading in the new host with exceptional rapidity (Banner and Lai, 1991).

The occurrence of three human CoV epidemics in less than 20 years, along with the emergence of less pathogenic human CoVs, arises some questions on how these viruses that have their reservoirs in bats and rodents may overcome the species barriers jumping to humans. The animal-to-human transmission of viruses has been already occurred in the past, but it seems that its frequency has been increased in the last decades, involving in a short time span not only CoVs, but also a plethora of genetically and biologically different viruses with zoonotic potential, such as Ebola virus, influenza viruses, flaviviruses, Hendra and Nipah viruses (McMahon et al., 2018). Climate changes that are intensifying in this first quarter of the 21st century are favouring the spread of vector-borne diseases through increasing the proliferation of vectors and predisposing to their occupation of new ecological niches. The emergence in temperate climate areas such as Europe of vector-borne diseases caused by viruses considered exotic until few years ago (West Nile virus, Usutu virus, Chikungunya virus) accounts for a progressive geographic expansion of tropical diseases thanks to the ongoing phenomenon of tropicalisation (McMahon et al., 2018). Deforestation and urbanization are other major factors that facilitate the spill-over of zoonotic agents to humans by reducing the habitat of wildlife and increasing the chances of contacts between wild animals (like bats, rodents and birds) and human beings (Beena and Saikumar, 2019; Lorusso et al., 2020). This could be the case of Ebola virus, Hendra and Nipah viruses, hantavirus and coronavirus infections. In addition, the close contact between human beings and different animal species sold at the wet markets of East Asia represents the optimal situation for the host species jump and adaptation to humans of potentially zoonotic agents like CoVs. It is not a coincidence that two of the most severe zoonoses of the last two decades (highly pathogenic H5N1 avian influenza and SARS) have emerged in the same Chinese province of Guangdong where the contact between humans and animals is closer (Lorusso et al., 2020).

### 3. Animal coronaviruses

#### 3.1. Coronaviruses in birds

Table 2 reports the most important avian CoV species recognised so far and their associated diseases. The number of avian species in which CoVs have been detected in the last years is humongous. Since the emergence of SARS-CoV in 2002, there has been increased interest in

| Avian species | CoV genus | CoV species | CoV subgenus | CoV common name | Associated disease |
|---------------|-----------|-------------|--------------|----------------|-------------------|
| Chicken (Gallus gallus domesticus) and other birds | Gammacoronavirus | Avian coronavirus | Igacovirus | Infectious bronchitis virus | Respiratory disease, kidney injury and/reproductive failures |
| Turkey (Meleagris gallopavo) | Gammacoronavirus | Avian coronavirus | Igacovirus | Turkey coronavirus (TCoV) | Enteric disease |
| Guinea fowl (Numida meleagris) | Gammacoronavirus | Avian coronavirus | Igacovirus | Guinea fowl coronavirus (GfCoV) | Enteric disease |
| Pheasant (Phasianus colchicus) | Gammacoronavirus | Avian coronavirus | Igacovirus | Pheasant coronavirus (PhCoV) | Enteric disease |
| Phasian (Pheasantus quennelli) | Gammacoronavirus | Avian coronavirus | Igacovirus | Phasian coronavirus (PhCoV) | Enteric disease |
CoVs in other species, including birds. Prior to that time, our knowledge of CoVs in avian species was limited largely to three birds of the order Galliformes, i.e., domestic fowl (Gallus gallus), turkeys (genus Meleagris) and pheasants (Phasianidae), with their infectious bronchitis virus (IBV), turkey coronavirus (TCoV), and pheasant coronavirus (PhCoV), respectively. These three viruses were considered for a long time different species for several reasons such as the diverse pathotype (enterotropic or respirotrophic), host range and genetic relatedness of the S protein (Cavanagh, 2007). This scenario radically changed after the discovery of several novel CoVs with high genetic diversity from different avian species and the novel rules for species designation of the Coronavirus Study Group (CSG https://talk.ictvonline.org/ictv-reports/ictv_9th_report/positive-sense-rna-viruses-2011/w/posrna_viruses/CoronavirusStudyGroup/). All these viruses as well as analogous IBV-like CoVs detected in other birds including penguins, pigeons, peafowl, parrots, waterfowl, teal, quail, duck and whooper swan (Cavanagh et al., 2002; Cercella et al., 2007; Domanska-Blicharz et al., 2014; Torres et al., 2013; Hughes et al., 2009; Liu et al., 2005; Wille et al., 2016; Jordan et al., 2015; Bande et al., 2016; Suryaman et al., 2019) have been assigned to the same viral species known as Avian coronavirus (AcCoV) within the subgenus Igacovirus of genus Gammacoronavirus.

IBV and IBV-like strains are commonly detected in both gallinaceous and non-gallinaceous birds, also asymptotically (Cavanagh, 2005). This might suggest that these species would act as wild reservoirs, spreading IBV strains over the last decades. IBV causes the infectious bronchitis (IB), a term adopted in 1931 for describing the main clinical characteristics of a transmissible respiratory disease of poultry detected for the first time in North Dakota (USA). IB has been now diagnosed worldwide and is one of the most important viral diseases of poultry characterised by respiratory signs, but it can also affect the kidneys and reproductive tract following viremia with a severity that differs depending on the involved viral strain (Cavanagh and Gelb, 2008). The disease also affects wild and ornamental birds (Liu et al., 2005; Chen et al., 2013).

IB control has been hampered by the intricate IBV evolution, which has been entailed, over the years, by the emergence of many different antigenic or genotypic types, commonly referred to as variants, with divergent molecular, biological, and antigenic properties. Being a CoV, IBV has, indeed, a considerable ability to change both by mutation and by homologous recombination events, which may cause, along with replicase stuttering or slippage, also insertions and deletions in the genome (Valastro et al., 2016). Through their S protein, IBV and IBV-like viruses recognise as cellular receptor the α2,3-linked sialic acid glycan, widely distributed in most avian species. This binding is in marked contrast to the α2,6-linked sialic acid glycan binding of IBV and IBV-like viruses (Ambepitiya Wickramasinghe et al., 2015b).

The S1 subdomain of a TCoV isolate from France in 2008 (TCoV-FR) had only 42 % sequence identity to that of the TCoV-US strain (Maurel et al., 2011). This diversity was biologically evident by the prominent tropism for the epithelium of the bursa of Fabricius and only mild tropism for the small intestine of turkey. TCoV-FR S1 protein did not show, instead, affinity for nonsialylated type 2 poly-LacNac (Ambepitiya Wickramasinghe et al., 2015a). This genetic diversity between TCoVs is in accordance with several recombination events involving IBVs on different continents with several unknown CoVs. On the one hand, the S genes of GfCoV/Fr/2011 (isolated in France in 2011) and TCoV-US share significant genetic relationships, and thus these viruses must have acquired their S gene from a common ancestor. On the other hand, GfCoV/Fr/2011 and Fr TCoV have a very similar genetic background in other genes. Two recombination events may be responsible for the genesis of TCoV-US and Fr TCoV. A first event occurred between an IBV EU recipient strain and an unknown AcCoV donor, resulting in a virus with a new S gene, whose evolution would have resulted in Fr TCoV and GfCoV/Fr/2011. A second recombination event involving a US IBV recipient and GfCoV/Fr/2011 would have generated US TCoV viruses, which share a stronger S gene similarity with GfCoV/Fr/2011 than with Fr TCoV (Brown et al., 2016).

Additional CoVs distinct from AcCoVs and mainly circulating in ducks (duck coronavirus, DgCoV), pigeons (pigeon coronavirus, PCoV), or geese (goose coronavirus, GCoV) have been identified (Cheng et al., 2013; Jonassen et al., 2005; Muradrasoli et al., 2010; Kim and Oem, 2014; Zhuang et al., 2015; Papineau et al., 2019). Although their genome seems to fulfill the official ICTV criteria required to distinguish a new species within the Gammacoronavirus genus, ICTV approval is still pending.

Historically, CoVs of birds were all included in the Gammacoronavirus genus and, in turn, all CoVs belonging to this genus were identified only in birds. However, this suggestion was rebutted by the evidence of a CoV belonging to the Gammacoronavirus genus in a beluga whale first discovered in 2008 (viral species Beluga whale coronavirus SW1 species, subgenus Cegacovirus, genus Gammacoronavirus) (Mihindukulasuriya et al., 2008), and of three novel CoVs, BuCoV HKU1, ThCoV HKU12, and MuCoV HKU13 in birds of the order Passeriformes, namely bulbuls (Pycnonotus jocosus), thrushes (Turdidae) and munias (Lonchura punctulata), respectively, which did not cluster phylogenetically with extant CoVs identified in birds. These latter three viruses were distinct from known CoVs forming a unique cluster in the phylogenetic tree, which was the basis for generation of the Deltacoronavirus genus (Woo et al., 2009). Importantly, additional novel viruses belonging to this novel genus were detected in wild birds (Woo et al., 2012; Chu et al., 2011; Durães-Carvalho et al., 2015; Torres et al., 2016). These viruses cluster with previously unclassified CoVs detected in various Asian carnivores, i.e., the Asian leopard cat (Prionailurus bengalensis) and Chines ferret badger (Nyctereutes procyonoides) (Dong et al., 2016).
CoVs belonging to the Betacoronavirus genus, which are strictly related to mouse hepatitis virus (MHV), were also described in wild birds, including parrots, in Brazil (Durães-Carvalho et al., 2015). Interestingly, this was not the first detection of viruses belonging to the Betacoronavirus genus in birds. Often overlooked is the discovery over 38 years ago of a CoV from the Manx shearwater (Puffinus puffinus), a bird that visits the shores of Britain in summer (Nuttall and Harrap, 1982; Cavanagh et al., 2007). This virus was also related to MHV. However, at that time, considering the unusual finding and that the virus was isolated by passage of shearwater material in the brains of mice, it was speculated that the detected virus was an MHV strain already present in the mice before inoculation (Cavanagh, 2007).

### 3.2. Coronaviruses in bats

Bats are an ancient and heterogeneous group of ecologically important mammals, representing nearly a quarter of all mammalian diversity on earth. They belong to the order Chiroptera and further classified in two suborders Yinpterochiroptera and Yangochiroptera. The first includes the non-echolocating Pteropodidae family (megabats) and five echolocating Rhinolophidae microbat superfamilies. Yangochiroptera contain thirteen echolocating microbat families (Tsagkogeorga et al., 2013). Bats are thought to host a large plethora of viruses. These include, amongst the others, lyssaviruses, filoviruses, henipaviruses, and reoviruses (Calisher et al., 2006).

Before SARS-CoV epidemic, bats were not known to host CoVs. Indeed, the first evidence of a bat CoV was published in 2005 (Poon et al., 2005). After the SARS epidemic, there was a boost in interest regarding searching for novel CoVs in various animals, including bats. To date, over 200 novel CoVs have been identified in bats and approximately 35 % of the bat virome sequenced to date is composed of CoVs (Chen et al., 2014). This data has been made available following the massive surveillance, coupled with the advent of next-generation sequencing (NGS) technology, which has been performed in wild animals (Woo et al., 2010; Banerjee et al., 2019). Just a small portion of these CoVs have been officially recognised by the ICTV; many others are still pending for official designation. CoV species detected in bats and officially recognised by the ICTV are listed in Table 3 and the following chapter reasonably discusses only officially recognized bat CoV species.

Bats can carry and transmit CoVs into local bat populations via migration even though little is known about the migratory patterns of these animals. Closely related CoVs can be detected in the same bat species living at locations separated by thousands of miles (Drexler et al., 2010) and different CoV species or genera can be found in different bat species living at the same roosting sites. However, some CoVs have been shown to be species-specific. Accordingly, regional patterns of bat CoV outbreaks at species level can be deduced from the population distribution of their respective bat hosts. Although bats seem to develop clinical diseases induced by several viruses and bacteria (Mühldorfer et al., 2011), generally CoVs do not cause apparently overt disease in these mammals, also experimentally. This phenomenon seems to be related with peculiar characteristics of their immune system (Ahn et al., 2019; Brook et al., 2020).

Based upon genomic data available so far, it is widely accepted that while birds represent the reservoir for CoVs belonging to genera Gammacoronavirus and Betacoronavirus, bats are the natural reservoir for alpha- and betacoronaviruses. However, only betacoronaviruses of subgenera Sarbecovirus, Merbecovirus, Nobecovirus and Hibeovirus have been detected in bats so far. Given that several betacoronaviruses from the subgenus Embecovirus have been discovered in rodents, it was speculated that rodent CoVs may be the ancestors of currently circulating viruses belonging to this subgenus (Wong et al., 2019). CoVs have been detected at high frequency in bats in all continents, with alphacoronaviruses being more widespread than betacoronaviruses (Wong et al., 2019).

Subgenus Colacovirus (genus Alphacoronavirus) officially comprises the viral species Bat coronavirus CDPHE15, so far composed by two bat CoVs strains named CDPHE15/USA/2006 and Myotis lucifugus CoV (MyCoV), which share a 98.2 % nucleotide identity across the whole genome. Both strains have been detected in bats. Myotis lucifugus bats (Vespertilionidae) also known as the northern American little brown bats. The former was detected in 2006 in Colorado (GenBank acc. no. KF430219), while the latter was reported in 2010 in Canada. This virus was identified in the intestines and lungs and associated with minimal pathology or inflammation (Subudhi et al., 2017). Subgenus Decacovirus (genus Alphacoronavirus) comprises the species Rhinolophus ferrumequinum alphacoronavirus HuB-2013 composed so far by BtMs-Alphacov/S2013 and BtRf-Alphacov/HuB2013 strains discovered in China in Myotis spp. and Rhinolophus ferrumequinum bats, respectively.

### Table 3

| CoV genus     | CoV subgenus | CoV species                                      | Common ancestor with/ Possible descendant | Reference             |
|---------------|--------------|--------------------------------------------------|-------------------------------------------|-----------------------|
| Alphacoronavirus| Colacovirus  | Bat coronavirus CDPHE15                           | Not determined                            | KF430219              |
| Alphacoronavirus| Decacovirus  | Rhinolophus ferrumequinum alphacoronavirus HuB-2013| Not determined                            | Wu et al. (2016)      |
| Alphacoronavirus| Decacovirus  | Bat coronavirus HKU10                            | Not determined                            | Woo et al. (2007); Lau et al. (2012) |
| Alphacoronavirus| Decacovirus  | Miniopterus bat coronavirus 1                     | Not determined                            | Poon et al. (2005); Chu et al. (2008) |
| Alphacoronavirus| Decacovirus  | Miniopterus bat coronavirus HK8                   | Not determined                            | Poon et al. (2005); Chu et al. (2008) |
| Alphacoronavirus| Myotacovirus | Myotis ricketti alphacoronavirus Sax-2011         | Not determined                            | Wu et al. (2016)      |
| Alphacoronavirus| Nyctacovirus | Nyctalus velatus alphacoronavirus SC-2013          | Not determined                            | Wu et al. (2016)      |
| Alphacoronavirus| Pedacovirus  | Scotophilus bat coronavirus S12                   | Porine epidemic diarrhoea virus           | Tang et al. (2006)    |
| Alphacoronavirus| Rhinacovirus | Rhinolophus bat coronavirus HKU2                  | Severe acute diarrhoea syndrome- coronavirus | Lau et al. (2005); Woo et al. (2006) |
| Alphacoronavirus| Setracovirus | NL63-related bat coronavirus strain BtYNL63 – 9b | Human coronavirus NL63                    | Tao et al. (2017)     |
| Betacoronavirus| Hibeovirus   | Bat Hb-beta coronavirus Zhejiang2013              | Not determined                            | Wu et al. (2016)      |
| Betacoronavirus| Merbecovirus | Middle East respiratory syndrome-related coronavirus | Not determined                            | Lelli et al. (2013); De Benedictis et al. (2014); Corman et al. (2014a, b) |
| Betacoronavirus| Merbecovirus | Pipistrellus bat coronavirus HKU5                 | Not determined                            | Woo et al. (2006)     |
| Betacoronavirus| Merbecovirus | Tylonycteris bat coronavirus HKU4                  | Not determined                            | Woo et al. (2006)     |
| Betacoronavirus| Nobecovirus  | Rousettus bat coronavirus GGCDC1                  | Not determined                            | Huang et al. (2016)   |
| Betacoronavirus| Nobecovirus  | Rousettus bat coronavirus HKU9                    | Not determined                            | Woo et al. (2007)     |
| Betacoronavirus| Sarbecovirus | Severe acute respiratory syndrome-related coronavirus | SARS-CoV-1; SARS-CoV-2 | Li et al. (2005); Lau et al. (2005) |

*Only viral species officially recognised by the International Committee on Taxonomy of Viruses are reported.*
These two viruses share very high sequence identities (higher than 98%), which dramatically decrease in the S genes (only 85% nucleotide identity) (Wu et al., 2016). The viral species *Bat coronavirus HKU10* (subgenus *Decacovirus*, genus *Alphacoronavirus*) was discovered in 2005 in China from *Rousettus* spp. and *Hipposideros* spp. bats. Additional strains from *Hipposideros* spp. bats were then discovered in 2006 and 2010 (Lau et al., 2012). A viral strain related to HKU10 was also identified in *Hipposideros pomona* in 2018 (GenBank acc. no. MN611523). Viral species *Miniopterus bat coronavirus 1* and *Miniopterus bat coronavirus HK8* belong to subgenus *Minunacovirus* of genus *Alphacoronavirus* and were discovered in 2005 immediately after SARS epidemic. CoVs belonging to *Miniopterus bat coronavirus 1* are commonly detected in *Miniopterus* spp. (Vespertilionidae) including *Miniopterus magnater* (Miniopterus bat coronavirus 1A) and *Miniopterus pusillus* (Miniopterus bat coronavirus 1B) from China, whereas *Miniopterus bat coronavirus HKU8* has been detected in *M. magnater*, *M. pusillus* and *Miniopterus schreibersii* (Poon et al., 2005; Chu et al., 2006, 2008). Infections of 1B and HKU8 were detected in seven *M. pusillus* specimens collected in 2004 and 2006 (Chu et al., 2008) but also in *Miniopterus* spp. bats in Kenya (Tong et al., 2009). *Myotis ricketti alpacacoronavirus Sax-2011* (subgenus *Myotacovirus*, genus *Alphacoronavirus*) and *Nyctalus velutinus alpacacoronavirus SC-2013* (subgenus *Nyctacovirus*, genus *Alphacoronavirus*) have been discovered in the last decade from samples collected in China from *Myotis ricketti* and *Nyctalus velutinus* bats (Vespertilionidae), respectively. *Scotophillus bat coronavirus 512* (subgenus *Pedacovirus*, genus *Alphacoronavirus*) has been first discovered in 2005 from samples of *Scotophillus kuhlii* (Vespertilionidae). Antibodies specific to the N protein of *Scotophillus bat coronavirus 512* have been also detected in serum collected from three bat species, namely *Scotophillus kuhlii*, *Miniopterus fuliginosus*, and *Rhinolophus monilis* (Chen et al., 2018). *BtCoV/S2005*, the representative strain of this viral species, likely has a common evolutionary precursor with porcine epidemic diarrhoea virus (PEDV) (Banerjee et al., 2019) and this latter, is thought to be originated from a cross-species jump of a BtCoV/S2005-like virus into pigs. A similar scenario is thought to have occurred with bat-CoV HKU2. This virus, identified for the first time from *Rhinolophus sinicus* (Lau et al., 2005; Woo et al., 2006) shares an 86% sequence identity with severe acute diarrhea syndrome-coronavirus (SADS-CoV) of pigs (Zhou et al., 2018). These two viruses are now included in the same viral species *Rhinolophus bat coronavirus HKU2* (subgenus *Rhinacovirus*, genus *Alphacoronavirus*). Viral strains *BtKYNL63-9a*, *BtKYNL63-9b*, *BtKYNL63-15* and *BtKYNL63-9a*, identified in 2010 in *Triaenops* afer bats from Kenya, form the viral species *NL63-related bat coronavirus strain BtKYNL63-9b* that is part of the subgenus *Scerotacovirus* (genus *Alphacoronavirus*) along with *Human coronavirus NL63* (Tao et al., 2017).

In this regard, a bat origin has been strongly suggested for two of the less-pathogenic HCoVs causing mild respiratory symptoms in immunocompetent people, namely HCoV-229E and HCoV-NL63, both less-pathogenic HCoVs causing mild respiratory symptoms in immunocompetent people, namely HCoV-229E and HCoV-NL63, both belonging to the *Alphacoronavirus* genus. Whereas HCoV-229E (subgenus *Davincovirus*) recognises as direct ancestor an alphacoronavirus from alpacas, which in turn derive from 229E-related CoVs identified in hippopotiderid bats (Corman et al., 2015), HCoV-NL63 is likely a recombinant virus originating from the distantly related 229E-related CoVs associated with hippopotiderid bats and CoVs associated with *Triaenops* afer bats (Tao et al., 2017) (Table 1). The S protein of HCoV-NL63 is more closely related to that of 229E-related CoVs, whereas the rest of the genome with CoVs included in the *NL63-related bat coronavirus strain BtKYNL63-9b* species (Tao et al., 2017). Different from the bovine coronavirus (BCoV)-like viruses that cause enteric disease, in 2007 a novel alpaca CoV was associated to respiratory disease in California, USA. Full-length genome analysis showed that this respiratory alpaca CoV was closely related to the alphacoronavirus HCoV-229E (subgenus *Davincovirus*) (Crossley et al., 2012). More recently, close relatives of HCoV-229E were detected in African hippopotiderid bats. Interestingly, both bat and alpaca viruses displayed an intact accessory gene ORF8 located at the genomic 3’ end, while HCoV-229E retained only a conserved TRS preceding remnants of this ORF, suggesting its loss after acquisition of a 229E-related CoV by humans. Therefore, HCoV-229 is likely a descendant of the alpaca alphacoronavirus (Corman et al., 2015).

Strains forming the viral species *Bat Hp-beta coronaviruses Zhejiang2013* (subgenus *Hibecovirus*, genus *Betacoronavirus*) were discovered in *Hipposideros pratti* bats from China in 2013 (Wu et al., 2016). Strain Ro-BatCoV GCCDC1 356 was identified from stools of *Rousettus leschenaultii*, a species of fruit bats (Pteropodidae) of southern Asia, which were collected in Yunnan province, China, in 2014 (Huang et al., 2016). Ro-BatCoV GCCDC1 356 shows a small intact ORF of 276 nucleotides embedded between the N and N7a genes. This ORF has no homology to any known coronavirus, and the encoded protein exhibited 54.9% amino acid identity with the p10 protein encoded by the first ORF of segment S1 of bat fusogenic orthoreoviruses (genus *Orthoreovirus*, species *Nelson Bay orthoreovirus*, also known as pteropine orthoreovirus). These viruses are double-stranded segmented RNA viruses, belonging to the family *Reoviridae*, and are able to cause severe pneumonia in humans (Chua et al., 2007; Lorusso et al., 2015). Ro-BatCoV GCCDC1 356 is included in the viral species *Rousettus bat coronavirus GCCDC1* within the subgenus *Nobecovirus*, genus *Betacor-

*Rousettus bat coronavirus HKU9*, belonging to subgenus *Nobecovirus*, was also identified in *Rousettus leschenaultii* and in other bat species (Mendenhall et al., 2017). This virus was first detected in 2007 in Guangdong province in China (Woo et al., 2007). Subsequent studies suggested that the virus was widely distributed and is circulating in different bat species (Ge et al., 2012). CoVs from the *BatKU9*-like cluster were also detected in *Hipposideros commersoni* and *Rousettus aegyptiacus* bats in Kenya (Tong et al., 2009). Being a fruit bat, *Rousettus leschenaultii* has a wider flying range than most of the insectivorous bats in China, thus it may carry viruses over long distances. A comparison of the reported HKU9-CoV sequences showed a high genetic diversity within this viral species (Luo et al., 2018a, b; Lau et al., 2010; Ge et al., 2012).

When MERS-CoV was first isolated in the Middle East in 2012 and its genome sequenced, it was found that it was most closely related to *Ty BatCoV HKU4 discovered in *Tylonycteris pachypus* and *Pi-BatCoV HKU5 discovered in *Pipistrellus abramus*, which were the only known members of subgenus *Merbecovirus* at that time. These two viruses are now the prototype strains of *Tylonycteris bat coronavirus HKU4* and *Pipistrellus bat coronavirus HKU5* viral species, respectively, within subgenus *Merbecovirus*, genus *Betacoronavirus*. Although MERS-related CoVs (MERS-CoVs) were laterly discovered, MERS-CoV was much closer in the S1 region to HKU4-CoV than to MERS-CoV or HKU5-CoV. Indeed, dipeptidyl peptidase 4 (DPP4), the receptor for MERS-CoV, is also the receptor for HKU4, but neither for HKU5 nor for early discovered MERS-CoVs. However, HKU4 prefers bat DPP4 over human DPP4, whereas MERS-CoV shows the opposite trend (Yang et al., 2014). So far, HKU4-CoVs are only carried by *Tylonycteris* spp. bats (*T. pachypus* and *T. robustula*) and are relatively conserved; HKU5-CoVs are found in different *Pipistrellus* spp. bats, including *P. abramus*, *P. pipistrellus* and *P. minus* (Fan et al., 2019).

Due to the current SARS-CoV-2 pandemic, attention should be given to the viral species *Severe acute respiratory syndrome-related coronavirus* (SARS-CoV, subgenus *Sarbecovirus*, genus *Betacoronavirus*) and *Middle East respiratory syndrome-related coronavirus* (MERS-CoV, subgenus *Merbecovirus*, genus *Betacoronavirus*), which enclose SARS-CoV and MERS-CoV, the first two highly pathogenic CoVs that were discovered in humans. In 2002, at the beginning of the SARS epidemic, almost all early human index patients had animal exposure in a market place, in Guangdong province, before developing disease. After SARS-CoV was identified, its RNA and/or specific antibodies were found in masked palm civets (*Paguma larvata*) and animal handlers in a market place. However, later investigations of farmed and wild-caught civets
revealed that SARS-CoV strains found in market civets were transmitted to them by other wild animals (Tu et al., 2004; Kan et al., 2005). Subsequently, novel CoVs related to human SARS-CoV (SARS-rCoVs) were discovered in horseshoe bats (genus *Rhinolophus*) in China and Hong Kong (Li et al., 2005; Lau et al., 2005). These SARS-rCoVs showed genome sequence identity of 88–90 % among themselves and 87–92 % identity to human or civet SARS-CoV isolates. SARS-rCoVs were detected in *Rhinolophus spp.*, bats of other regions of China (Tang et al., 2006; Woo et al., 2006; Yuan et al., 2010; Ge et al., 2013). SARS-rCoVs with higher genetic diversity with respect to Chinese strains were also detected in rhinolophid bats from Slovenia, Bulgaria and Italy in Europe (Drexler et al., 2010; Rihtaric et al., 2010; Balboni et al., 2011). CoVs related to SARS-rCoV were also detected in *Hipposideros* spp. and *Chaerophon* spp. bats from Ghana, Kenya and Nigeria (Hu et al., 2015). These evidences suggested that bats may be the natural hosts for SARS-CoV and that wild carnivores were only intermediate hosts. Although these SARS-rCoVs showed high sequence identity to SARS-CoV, they were demonstrated to be unable to bind to the human cell angiotensin converting enzyme II (ACE2) receptor, the receptor of SARS-CoV, as a consequence of deletions in their S protein (Ren et al., 2008). Besides, the theory of bat origin of SARS-CoV lacked a powerful support due to the failure of direct isolation of this virus from bats. Thus, considering that no direct progenitor of SARS-CoV was found in bats and that RNA recombination is the fuel for CoV evolution, it has been proposed that SARS-CoV emerged through recombination of bat SARS-rCoVs. This hypothesis was made after the evidence of a single bat cave in Yunnan, China, with very high CoV diversity and considering that, within the identified CoVs, all genetic elements needed to form SARS-CoV have been identified in that single cave (Ge et al., 2013). Recombination analysis also strongly supported the hypothesis that the civet SARS-CoV strain S23 originated following a recombination event of two existing bat strains, WIV16 and R4/492 (Hu et al., 2017). Moreover, WIV1, the closest relative to SARS-CoV that has been found in bats so far (more than 95 % nucleotide identity, higher than that of any other bat SARS-rCoVs (76–92 %)), likely arose through recombination of two other prevalent bat SARS-rCoV strains. The most frequent recombination breakpoints were within the S gene and upstream of ORF8, which encodes an accessory protein. These genes were also involved in the crucial adaptation pathways of SARS-CoV from bats to wild carnivores, from wild carnivores to humans, and from human to human (Cui et al., 2019). WIV1 has been shown to have the capacity to bind to the human, civet and bat cell ACE2 receptor (Ge et al., 2013). The isolation in cell-culture of a highly related SARS-CoV strain, coupled with the evidence of a functional S protein capable of using the same ACE2 receptor, provided robust and conclusive evidence for the bat origin of SARS-CoV. An additional SARS-rCoV strain has been shown, by reverse genetics studies, to have the capacity to bind to the human ACE2 receptor (Menachery et al., 2015).

Quite the opposite, a direct bat CoV highly related to MERS-CoV of humans was never detected. Indeed, the genome sequences of MERS-CoV in humans and dromedaries possess only around 65–80 % nucleotide identities to those of the other members of subgenus *Merbecovirus* from different bats. Human MERS-CoVs were instead almost identical to MERS-CoVs identified in dromedary camels (*Camelus dromedaries*). Lately, genomic sequence analyses indicated that CoVs new belonging to the MERS-rCoV species were found in several bat species from two bat families, *Vespertilionidae* and *Nycteridae* (Lelli et al., 2013; De Benedictis et al., 2014; Corman et al., 2014a, b; Anthony et al., 2017; Moreno et al., 2017; Wong et al., 2019). However, none of these MERS-rCoVs is a direct progenitor of MERS-CoV, as their S proteins differ substantially from that of the human virus. The closest relative to MERS-CoV of humans and dromedary camels is MERS-rCoV strain *Neoromicia*/*5038* isolated from *Neoromicia capensis* bats in South Africa (Geldenhuys et al., 2018, Table 1). A short sequence (around 200 nucleotides) of viral RNA identical to that of MERS-CoV was also detected in a *Taphozous perforates* bat in Saudi Arabia (Memish et al., 2013). Overall, although it is widely accepted that MERS-CoV ancestor is in bats, further studies are warranted in order to discover the precise mechanisms of its emergence in dromedary camels and humans. It was suggested that MERS-CoV ancestors had been circulating in bats for very long time. MERS-CoV has evolved to adapt to use human receptor and the DPP4-recognising bat coronaviruses like HKU4 may follow up, thereby posing a serious risk to human health. Recent MERS-rCoVs were shown to have the capacity to bind to the DPP4 as entry cell receptor as they acquired the S1 through recombination with HKU4-like viruses (Luo et al., 2018a, b).

As for the recent and threatening COVID-19 outbreak in humans, we certainly know that SARS-CoV-2 belongs to the species SARS-rCoV together with SARS-CoV from humans and SARS-rCoVs from wild carnivores and horseshoe bats (genus *Rhinolophus*) (Gorbalenya et al., 2020; Zhou et al., 2020; Wu et al., 2020). Epidemiological investigations revealed that many initial patients were exposed to wildlife at the Huanan seafood wholesale market (South China Seafood Market), which is the largest seafood market in central China (Lorusso et al., 2020). SARS-CoV-2 has been assigned to an existing species of hundreds of known viruses largely isolated from bats. These viruses have names derived from SARS-CoV, but only the viral isolates originating from the 2002–2003 outbreak have been confirmed to cause SARS in humans (Gorbalenya et al., 2020). Importantly, it has also been confirmed that SARS-CoV-2 uses the ACE2 receptor through the receptor binding domain (RBD) of the S protein (Hofmann et al., 2020; Zhou et al., 2020). Likely, also SARS-CoV-2 has a bat origin. According to genome sequences available so far, the most closely related virus (96.2 % of nucleotide sequence identity) to SARS-CoV-2 is strain BatCoVRaTG13 identified from a bat, *Rhinolophus affinis*, from Yunnan province, China, followed by SARS-rCoVs identified from pangolins (Tang et al., 2020). The receptor-binding spike protein of SARS-CoV-2 is highly divergent from other CoVs with less than 75 % nucleotide sequence identity to all previously described SARS-rCoVs, except for a 93.1 % nucleotide identity to *BatCoV* RaTG13 (Zhou et al., 2020). Although SARS-CoV-2 uses the ACE2 receptor, five out six critical amino acid residues in RBD were different between SARS-CoV-2 and SARS-CoV; the same residues were instead identical to those of pangolin SARS-rCoVs and, in turn, only one of these residues was identical to those of BatCoV RaTG13 (Tang et al., 2020), although this latter shows the highest nucleotide sequence identity with SARS-CoV-2 along the whole genome. Thus, it was tempting to speculate that SARS-CoV-2 RBD region might have originated from recent recombination event in pangolins or that SARS-CoV-2 and SARS-rCoVs of pangolins represent the result of coincidental evolution (Lam et al., 2020; Tang et al., 2020). Overall, it remains to be solved whether also SARS-CoV-2 needed an intermediate (and amplification) host before being able to infect humans as it was the case for SARS-CoV and other HCoVs. Since a mammal reservoir has not yet been identified, a prudent use of specific antigens is strongly recommended for serological diagnosis of SARS-CoV-2 in animals as cross-reactions with viruses of the *Alphacoronavirus* genus, widespread in animals, might occur (Sun and Meng, 2004). 3.3. Coronaviruses in rodents

Analogously to bats, but with a lesser extent, also rodents have been recently demonstrated to play a significant role in the evolution of CoV, in particular of those belonging to subgenus *Embecovirus* of genus *Betacoronavirus*. *Rodentia* (rodents) is the largest order of mammals with more than 2000 species worldwide, representing a major source of zoonotic infectious diseases (Han et al., 2015). For decades, only one species of coronavirus, *Murine coronavirus* (subgenus *Embecovirus*, genus *Betacoronavirus*), has been associated with rodents. The prototype virus, which was named mouse hepatitis virus (MHV), was first isolated in mice in 1949 (Cheever et al., 1949). A MHV variant was laterly identified in rats in 1970 (Parker et al., 1970). Rat coronavirus (pCoV) causes epidemics of respiratory disease in laboratory rat colonies. The two
Table 4

| Coronavirus in domestic swine and associated diseases. | CoV genus | CoV subgenus | CoV species | Possible ancestor | Associated disease | Reference |
|-------------------------------------------------------|-----------|--------------|-------------|------------------|--------------------|-----------|
| Transmissible gastroenteritis virus (TGEV)            | Alphacoronavirus | Tegacovirus | Alphacoronavirus | Canine coronavirus | Gastroenteritis | Doyle and Hutchings (1946) |
| Alphacoronavirus Tegacovirus                           | Porcine respiratory coronavirus (PRCoV) | Pedacovirus | Porcine epidemic diarrhoea | Gastroenteritis | Wood (1977) |
| Alphacoronavirus Pedacovirus                           | Porcine epidemic diarrhoea virus (PEDV) | Common bat ancestor with Scotophilus | Gastroenteritis | Wood (1977) |
| Alphacoronavirus Rhinacovirus                          | Severe acute diarrhoea syndrome-coronavirus (SADS-CoV) | Common bat ancestor with Rhinolophus | Gastroenteritis | Gong et al. (2017) |
| Betacoronavirus Embecovirus                            | Betacoronavirus-1 | Porcine haemagglutinating encephalomyelitis virus (PHEV) | Enteric disease | - |
| Betacoronavirus-1                                    | Coronavirus | Coronavirus HKU15 | Coronavirus HKU15 | - | - |
| Deltacoronavirus Buldecovirus                         | Coronavirus | Coronavirus HKU15 | Coronavirus HKU15 | - | - |

 prototype strains of RCoV are sialodacryoadenitis virus (SDAV) and Parkers's RCoV (RCoV-P) (Bhatt et al., 1972; Parker et al., 1970). Both strains infect the respiratory tract, and SAV can also infect the eye, salivary and lacrimal glands. Young rats are especially susceptible to RCoV with the infection occurring in the lower respiratory tract and developing into interstitial pneumonia (Parker et al., 1970).

Together with feline infectious peritonitis virus (FIPV) and IBV, MHV has been one of the most strictly animal CoV studied ever. MHV is a natural pathogen of mice, normally infecting the liver, gastrointestinal tract, and central nervous system, causing a wide range of disease, including hepatitis, gastroenteritis, and acute and chronic encephalomyelitis. Importantly, it served as model for CoV replication and pathogenesis, with emphasis for neuro-invasion and neurovirulence (Weiss and Navas-Martin, 2005). As for the additional structural protein HE, some strains (such as JHM) of MHV contain the HE protein, while others (such as A59) do not (Shieh et al., 1989; Yokomori et al., 1989).

The role of rodents in the evolution of CoVs belonging to embecoviruses has been recently highlighted by means of the discovery of a novel betacoronavirus in Norway rats (Rattus norvegicus) in China. This virus forms a separate species named China Rattus coronavirus HKU24 (ChRCoV HKU24) within the Embecovirus subgenus. Although designated as a novel species, this virus possessed genome characteristics that resemble to those of both Betacoronaviruses-1 and Murine coronavirus, suggesting that ChRCoV HKU24 represents the murine origin of Betacoronaviruses-1, with interspecies transmission from rodents to other mammals having occurred centuries ago (Lau et al., 2015).

Genus Betacoronavirus consists of five subgenera, with bat CoVs being included in all but one of subgenus Embecovirus, where rodent, human and bovine CoVs are included (https://talk.ictvonline.org/taxonomy/). This supports the hypothesis that rodent CoVs were the ancestors of embecoviruses of other animals, while bats are the natural reservoirs for all other betacoronaviruses. Importantly, rodent CoVs are not restricted to genus Betacoronavirus. A deep virological screening was performed in 1465 rodents sampled in Zhejiang province, China, during 2011–2013, with nearly 2% of rodents testing positive for CoV (Wang et al., 2015). In particular, CoVs were detected in 10 striped field mice (Apodemus agrarius), 4 Norway rats, 14 lesser ricefield rats (Rattus norvegicus), 1 Asian house rat (Rattus tanezumi) and 1 Chinese white-bellied rat (Niviventer confucianus). Amplicons of the replicate gene sequences were recovered from 21 (70 %) of the CoV RNA positive rodent samples described above and whole genome or nearly whole genome sequences (> 98 %) were recovered from 1 and 4 CoV positive samples, respectively. By means of whole genome sequence analysis, authors were able to identify a divergent alphacoronavirus, which was newly officially designated as species Lucheng Rn rat coronavirus (LRNV) within the subgenus Luchacovirus, and two novel betacoronaviruses termed Longquan Aa mouse coronavirus (LAMV) and Longquan RI rat coronavirus (LRIV) and assigned to the two established species Betacoronaviruses-1 and Murine coronavirus, respectively (Wang et al., 2015). Moreover, LRNV seems to be a recombinant virus as its N protein gene is more closely related to those of the genus Betacoronavirus. Overall, the discovery of rodent-associated CoVs belonging to subgenera that are distinct from those including bat CoVs warrants further investigations upon the role played by rodents in the evolution and emergence of these viruses.

SARS-CoV replication has been studied in mice, Syrian golden and Chinese hamsters. The most severe symptoms of SARS were observed in aged animals. Indeed, aged mouse model of SARS-CoV has been generated (Gretebeck and Subbarao, 2015). Transgenic mice expressing human ACE2 were also developed to closely mimic SARS-CoV infection in humans. Some animal models have been tested and analysed on the genomic and proteomic level to study the pathogenesis of SARS-CoV. Therefore, we have reason to believe that such models would work also for SARS-CoV-2. Quite the opposite, studies have demonstrated that mice, guinea pigs and hamsters are not susceptible to experimental MERS-CoV infection, mainly because their homologous DPP4 molecules
do not function as receptors for MERS-CoV entry (Cockrell et al., 2014). The first mouse model of MERS infection reported in 2014 involved transducing animals with recombinant adenovirus 5 encoding human DPP4 (hDPP4) molecules intranasally, and this resulted in replication of MERS-CoV in the lungs. This mouse model also showed clinical symptoms of interstitial pneumonia, including inflammatory cell infiltration, and thickened alveolar and mild oedema (Song et al., 2019).

3.4. Coronaviruses in swine

Currently, six CoVs are circulating in swine (Table 4). These include four alphacoronaviruses, transmissible gastroenteritis virus of swine (TGEV) and its derivative porcine respiratory coronavirus (PRCoV) (subgenus Tegacovirus), porcine epidemic diarrhoea virus (PEDV) (subgenus Pedacovirus) and SADS-CoV (subgenus Rhinacovirus), one betacoronavirus, porcine haemagglutinating encephalomyelitis virus (PHEV) (subgenus Embe covirus), and one deltacoronavirus, porcine deltacoronavirus (PDCoV) (subgenus Buldevir avirus). TGEV, PEDV, SADS-CoV and PDCoV are responsible for acute gastroenteritis in swine, with fatal infections in piglets born to seronegative sows, PRCoV causes a mild respiratory disease and PHEV is the causative agent of neurological and/or digestive disease in pigs (Mora-Diaz et al., 2019; Wang et al., 2019).

TGEV was first described in UK in 1950s, representing the oldest known swine CoV. TGEV and PRCoV are closely related to canine coronavirus (CCoV) and feline coronavirus (FCoV) forming with these coronaviruses the four alphacoronaviruses, transmissible gastroenteritis virus of swine (TGEV) which are strictly related in the S gene, have only remnants of the N-terminal domain-deletion (NTD-del) strains that are G2-like strains containing a 194 to 216-aa deletion within the N-terminal domain of the S1 subunit, also associated to mild clinical forms (Hou and Wang, 2019). Recombinant strains between PEDV and TGEV have been also reported in Europe (Akimkin et al., 2016; Belsham et al., 2016; Boniotti et al., 2016).

SADS-CoV, now referred to as swine enteric alphacoronavirus (SeACoV), is another virulent swine enteric alphacoronavirus that originated from bats, sharing an 86 % sequence identity with a bat alphacoronavirus HKU2-CoV. Since viruses displaying a 96–98 % sequence identity to SADS-CoV were detected in Rhinolophus spp bats, SADS-CoV and HKU2-CoV likely descend from a common ancestor (Zhou et al., 2018). Accordingly, both viruses now belong to the unique species Rhinolophus bat coronavirus HKU2.

In contrast, PHEV, which was first described in 1957 in nursery pigs with encephalomyelitis in Ontario, Canada, has not derived from bat CoVs, but its evolutionary history is tightly intermingled with other two closely related betacoronavirus, HCoV-OC43 and the oldest known BCoV, with which PHEV may have common ancestors (Vijgen et al., 2006) and is included in the same viral species, Betacoronavirus-1 (Corman et al., 2018). Most probably, HCoV-OC43 and PHEV descend from a rodent betacoronavirus through preliminary adaptation to BCoV, from which they may have emerged in the context of a pandemic recorded historically at the end of the 19th century (Corman et al., 2018).

PDCoV was recently detected in 2012 in Hong Kong during CoV molecular surveillance in avian and mammalian species. This swine deltacoronavirus seems to recognise another different ancestor, likely emerging from a host-switching event between avian and mammal CoVs. The most closely related PDCoV relative has been identified in quail deltacoronavirus UAE-HKU30 and the virus has been proposed to be a recombinant between two other avian deltacoronaviruses, sparrow CoV HKU15 and bulbul CoV HKU11. All these deltacoronavirus are now members of the same species Coronavirus HKU15 (Lau et al., 2018).

Pigs were found to be susceptible to experimental infection with the betacoronavirus MERS-CoV (Vergara-Alert et al., 2017), while SARS-CoV RNA was detected in pigs and wild boars (Chen et al., 2005; Wang et al., 2005). In contrast, a recent experimental infection demonstrated that pigs are not susceptible to SARS-CoV-2 (Shi et al., 2020).

Few studies have been carried out to assess the circulation of CoVs in farmed or free-ranging wild boars (Sus scrofa). Antibodies against TGEV/PRCoV were detected in some animals in Slovenia (Vengust et al., 2006) and Croatia (Roic et al., 2012) and PEDV RNA was demonstrated in South Korea (Lee et al., 2016). A wild boar sold at a live animal market of Guangzhou, China, was positive for SARS-CoV RNA (Wang et al., 2005).

3.5. Coronavirus in ruminants

The main CoVs infecting ruminants are reported in Table 5. The oldest known ruminant CoV is BCoV, which is also the prototype of the species Betacoronavirus-1 (subgenus Embe covirus, genus Betacor onavirus). This virus is able to cause a variety of clinical forms, including enteric disease with high mortality rates in neonate calves, winter disease (a severe enteric form) in lactating cows (Decaro et al., 2008a). It was postulated that the presence of genetic signatures differentiates enteric and respiratory BCoVs (Hasokuzu et al., 1999), but it was ultimately evident that the same virus strain could be responsible for simultaneous appearance of enteric and respiratory disease in the same animals (Chouljenko et al., 2001).

It has been postulated that BCoV originated from a rodent CoV (Corman et al., 2018). Very recently, a novel CoV, representing a new viral species, referred to as China Rattus coronavirus HKU24 (ChRCoV-HKU24), was detected in Norway rats in China. This virus was phylogenetically distinc from MHV and HCoV-HKU1 and displayed genome
| Ruminant species | CoV genus | CoV species | CoV common name | Reference |
|------------------|-----------|-------------|----------------|-----------|
| *Bos taurus*     | Betacoronavirus | Embecovirus | Betacoronavirus-1 (CoV-1) | China Rattus coronavirus HKU24 |
|                  |           |             | Bovine coronavirus (BCoV) | Kaye et al., (1975) |
| *Ovis aries*     | Betacoronavirus | Embecovirus | BCoV-like coronavirus | BCoV-like coronavirus BCoV | Tzipori et al., (1978) |
| *Lama lama*      |           |             | BCoV-like coronavirus | BCoV-like coronavirus BCoV | Muñoz et al., (1996) |
| *Lama pacos*     |           |             | BCoV-like coronavirus | BCoV-like coronavirus BCoV | Cebra et al. (2003) |
| *Camelus dromedarius* |           |             | Dromedary camel coronavirus UAE-HKU-23 (DcCoV UAE-HKU23) | Dromedary camel coronavirus UAE-HKU-23 (DcCoV UAE-HKU23) | Woo et al., 2014 |
| *Alpaca*         | Alphacoronavirus | Duvinacovirus | Human coronavirus 229E | Alpaca (alpha)coronavirus (ACoV) | African hypposiderid bat coronavirus | Crossley et al. (2012) |
| *Dromedary camel* | Alphacoronavirus | Duvinacovirus | Human coronavirus 229E | Dromedary camel alphacoronavirus | Human coronavirus 229E | Crossley et al. (2012) |

**Table 5**

Coronaviruses in domestic and domesticated ruminants and associated diseases.

**Features**

- BCoV is paradigmatic of how CoVs are able to cross the interspecies barriers, establishing its derivatives as separate viral lineages affecting the respiratory and/or enteric tract of humans (HCoV-OC43), swine (PHEV), horses (equine coronavirus, ECoV), and dogs (canine respiratory coronavirus, CRCoV). A number of BCoV-related viruses, all currently included in the unique species *Betacoronavirus-1*, have been detected in the enteric and/or respiratory tract of domestic and wild ruminants. These BCoV-like CoVs include viruses of domestic and domesticated ruminants that were reported in sheep and goats (Reinhardt et al., 1995; Yang et al., 2008), water buffalo (*Bubalus bubalis*) (Decaro et al., 2008c), llamas (*Lama lama*) and alpacas (*Vicugna pacos*) (Cebra et al., 2003; Jin et al., 2007). In the wild, BCoV-like CoVs were demonstrated in six species of the *Cervidae* family, which are caribou/reindeer (*Rangifer tarandus caribou*), elk/wapiti (*Cervus elephas*), sambar deer (*Cervus unicolor*), white-tailed deer (*Odocoileus virginianus*), sika deer (*Cervus nippon yesoensis*) and water deer (*Hydropotes inermis*) (Amer, 2018). Similar viruses were also found to circulate in the giraffe (*Giraffa camelopardalis*) (Hasokuzu et al., 2007), several species of antelopes (Aleksseev et al., 2008; Chung et al., 2011), and dromedary camels (*Camelus dromedarius*) (Woo et al., 2014). The last strain, detected in the United Arab Emirates and consequently named dromedary camel coronavirus UAE-HKU-23 (DcCoV UAE-HKU23), was slightly divergent from other BCoV-like viruses (Woo et al., 2014).

Dromedary camels are susceptible to MERS-CoV infection, developing asymptomatic infections or mild upper respiratory disease, so that they are considered the natural host of MERS-CoV, with adult animals in many countries in the Middle East as well as in North and East Africa showing > 90% seroprevalence to the virus (Hemida et al., 2017b). Although human-to-human transmission has occurred outside Middle East due to travel-associated patients with MERS and has caused large clusters of human cases within healthcare facilities in Saudi Arabia, Jordan and United Arab Emirates, it remains inefficient and sustained community transmission has not been documented so far, thus suggesting multiple virus introduction into the human population by infected dromedaries (Hemida et al., 2017b). More recently, a phylogenetic study of 173 MERS-CoV full-genome sequences revealed recombination signatures that defined five major phylogenetically stable lineages, all of which contained human and camel MERS-CoV sequences (Sabir et al., 2016). In the same study, an alphacoronavirus strictly related to HCoV-229E was found in the respiratory tract of dromedary camels of Saudi Arabia (Sabir et al., 2016). Although some studies ruled out the susceptibility of other domestic ruminants to MERS-CoV (Reusken et al., 2013; Adney et al., 2016), a recent study detected specific antibodies and RNA in sera and nasal secretions, respectively, of domestic ruminants raised in Africa, including sheep, goats and cattle (Kandell et al., 2019). Llamas were found to be susceptible to experimental infections with MERS-CoV (Vergara-Alert et al., 2017).

**3.6. Coronaviruses in equines**

The only CoV that has been so far known in horses is ECoV, which is a BCoV-descendant betacoronavirus (subgenus *Embecovirus*). ECoV was first isolated from the faeces of a diarrhoeic foal in 1999 (ECoV-NC99) in North Carolina, USA (Guy et al., 2000), and was initially believed to only affect foals. Since 2010, the virus has been recognised in Japan, Europe and the USA as a new, clinically important, enteric virus of adult horses (Pusterla et al., 2018).

Despite MERS-CoV was successfully adapted to the *in-vitro* growth in equine cell lines (Meyer et al., 2015), serological and molecular
investigations have demonstrated that horses are not naturally infected by MERS-CoV (Meyer et al., 2015; Hemida et al., 2017a), nor they are susceptible to experimental infection (Adney et al., 2016; Vergara-Alert et al., 2017). However, surprisingly, MERS-CoV RNA was detected in respiratory specimens of three donkeys of 42 from Egypt (Kandeil et al., 2019), a finding that requires further confirmation.

A molecular survey aimed to assess CoV circulation in horses in Saudi Arabia and Oman has detected two DcCoV UAE-HKU23 strains in enteric samples of horses (Hemida et al., 2017a).

Scarce data are available about CoV circulation in donkeys. These equids are susceptible to ECoV infection since positive RT-PCR results were obtained from a donkey in Ireland (Nemoto et al., 2019). In addition, three donkeys (7.1%) of 42 from Egypt tested positive for MERS-CoV RNA in their nasal secretions (Kandeil et al., 2019).

### 3.7. Coronaviruses in carnivores

CoVs of carnivores are listed in Table 6. Three CoVs are known in dogs, i.e., two alphacoronaviruses of the subgenus *Tegacovirus*, namely CCoV-I and CCoV-II, and one betacoronavirus of the subgenus *Embecovirus*, namely CRCoV.

*CCoVs* (species *Alphacoronavirus-I*) are commonly responsible for mild, self-limiting enteritis in pups (Decaro and Buonavoglia, 2008). Although they are neglected viruses and vaccination is not recommended due to the absence of an effective challenge model, two independent studies have demonstrated their significant involvement in the onset of acute canine enteritis (Duijvestijn et al., 2016; Dowgier et al., 2017). The evolutionary history of CCoVs is tightly intermingled with that of TGEV and FCoVs. CCoV-I possesses a divergent spike protein and the intact form of an additional gene, ORF3, whose remnants are present in CCoV-II and, at a lesser extent, in TGEV. Therefore, CCoV-II has likely emerged as a consequence of recombination between the original CCoV-I and an unknown CoV in the S gene and of progressive loss of ORF3 (Lorusso et al., 2008). A further recombination occurred in the very 5’ end of the S gene between CCoV-II and TGEV, giving rise to back recombinant CCoV-II strains, also known as TGEV-like CoVs, having a spike protein N-terminus of TGEV in a CCoV-II backbone (Decaro et al., 2009, 2010). Consequently, the CCoV taxonomy was revised, with classical and TGEV-like strains being referred to as CCoV-Ia and CCoV-Ib, respectively. While CCoVs are usually involved in mild forms of diarrhoea, there are some hypervirulent strains that are associated to severe, haemorrhagic, sometimes fatal gastroenteritis. In addition, CCoV-IIa strains, designated panotropic CCoV, that are able to spread systemically and cause severe disease and the death of infected dogs have been reported in Italy (Buonavoglia et al., 2006; Alfano et al., 2020), other European countries (Decaro et al., 2013) and South America (Pinto et al., 2014). Genomic sequences from panotropic CCoVs were analysed, but no obvious genetic signatures that may have caused the switch in pathogenicity were found (Decaro and Buonavoglia, 2011; Decaro et al., 2013).

Different from CCoV-I and CCoV-II, the betacoronavirus CRCoV is associated with mild respiratory signs and has been proposed as an etiological agent of canine infectious respiratory disease (CIRD) together with other viral and bacterial agents (Decaro and Buonavoglia, 2008). The virus was first detected firstly in UK in 2003 (Erles et al., 2003) and subsequently in other European and extra-European countries (Decaro et al., 2007, 2016; Mitchell et al., 2017; Maboni et al., 2019; Piewbang et al., 2019; More et al., 2020). Being a BCoV derivative, CRCoV possesses the same genomic organisation, with some differences in accessory ORFs located between the S and E protein genes. In particular, while some CRCoVs possess a unique 8.8 kDa protein gene directly downstream of the S protein gene, other canine BCoV-like CoVs display the canonical set of BCoV accessory genes but with truncated forms of the 4.8 kDa protein gene (Lorusso et al., 2009).

In cats, two *Alphacoronavirus-I* genotypes are known, namely FCoV type I (FCoV-I) and FCoV type II (FCoV-II), the latter being generated as...
a consequence of recombination events between CCoV-II and FCoV-I that generated viruses with a CCoV-II genomic region, encompassing ORF1b, ORF2 (S gene), ORF3abc, ORF4 (E gene), and partial ORF5 (M gene), in the context of an FCoV-I backbone (Pedersen, 2014). Both genotypes are involved in the development of feline infectious peritonitis (FIP), a perivascular pneumonitis of cats that may occur in two clinical forms, effusive and non-effusive FIP, which are characterised by prevalence of effusions in the body cavities and of pyo-granulomatous lesion in organs, respectively. FIP occurs as a consequence of a change in tissue tropism of an enteric FCoV strain (feline enteric coronavirus, FECV), infecting enterocytes of the intestinal villi, that acquires the ability to infect monocytes/macrophages switching to the more virulent FIPV, which is responsible for systemic infections and dysregulation of the proinflammatory cytokines (Addie et al., 2009).

The changes responsible for the pathogenetic shift have been investigated for many decades, being suggested to be variably represented by point mutations located in the S gene (Rottier et al., 2005), deletion/insertion in the group-specific genes 3c and E genes between distinct coronaviruses (Koike et al., 2016). Wis et al. (2010) have shown that FRECV and FRSCV differ significantly in spike protein and that deletions in FRCoV 3c may also correlate with the severe pathotype of FRSCV. Recombination in the S, M, and E genes between different FCoVs has been also reported (Lamers et al., 2016).

Different FCoVs were found to circulate in wild carnivores. CCoVs were detected in wolves (Canis lupus), red foxes (Vulpes vulpes), Eurasian otters (Lutra lutra), common genets (Genetta genetta) (Alfano et al., 2019; Rosa et al., 2020). CCoV-like viruses were also found in African wild carnivores, including spotted hyenas (Crocuta crocuta) and silver-backed jackals (Canis mesomelas) (Goller et al., 2013). FCoVs have a wide circulation in non-domestic felids (Kennedy et al., 2002, 2003), with FIP cases being reported in servals (Felin serval) (Juan-Salles et al., 1997), cheetahs (Acinonyx jubatus) (Kennedy et al., 2001b), mountain lion (Puma concolor) (Stephenson et al., 2013), and European wildcat (Felin silvestris) (Watt et al., 1993).

Divergent Alphacoronavirus-1 viruses were detected in Chinese ferret badger (Nectereutes procyonoides) and raccoon dog (Melagole moschata) (Dong et al., 2007). The same study reported the identification in Asian leopard cat (Prionailurus bengalensis) and Chinese ferret badger of an unclassified CoV, which was closely related to gammacoronaviruses in most parts of the genome, whereas the S gene displayed the highest sequence identity to alphacoronaviruses (Dong et al., 2007). With the discovery of deltacoronaviruses, these viruses were later included in this novel genus along with avian and porcine strains (Woo et al., 2009; Wang et al., 2014).

Some domestic and wild carnivores are also susceptible to SARS-CoV infection. While the potential natural reservoirs are horsehoe bats, SARS-like CoV strains were found to be widespread in masked palm civets (Paguma larvata) and raccoon dogs, which were suspected to be intermediate hosts (Guan et al., 2003). Full-genomic comparative analysis has shown that SARS-like CoVs isolated from palm civets are under strong selective pressure and are genetically most closely related to SARS-CoV strains infecting humans early in the outbreaks (Song et al., 2005). Sequence analysis of the SARS-CoV-like virus in masked palm civets indicated that they were highly homologous to human SARS-CoV with nucleotide identity over 99.6 %, indicating the virus has not been circulating in the population of masked palm civets for a very long time (Shi and Hu, 2008). A Chinese ferret-badger (Melagole moschata) was found to have neutralising antibodies against SARS-CoV (Guan et al., 2003), whereas SARS-CoV RNA was detected in naturally infected cats and red foxes (Vulpes vulpes), but not in domestic dogs (Wang et al., 2005). There was, however, a single dog testing positive for SARS-CoV (https://apps.who.int/iris/bitstream/handle/10665/70863/WHO_CDS_CSR_GAR_2003.11_eng.pdf). Among carnivores, SARS-CoV-2 is able to infect cats, ferrets and, at a lesser extent, dogs (Shi et al., 2020).

3.8. Coronaviruses in other species

In 2008, a highly divergent CoV, tentatively named SW1, was discovered in a deceased beluga whale (Delphinapterus leucas) with pneumonia and hepatic necrosis (Mihindukulasuriya et al., 2008). The virus was only distantly related to RV, so that it now represents the prototype of the single mammalian CoV species belonging to the genus Gammacor- onavirus, namely Beluga whale coronavirus SW1 (BWCW-SW1) (subgenus Cecacovirus). Few years later, related gammacoronaviruses were retrieved from faecal samples of three Indo-Pacific bottlenose dolphins (Tursiops aduncus), which were named bottlenose dolphin CoV (BdCoV) HKU22. Comparative genome analysis showed that BdCoV-HKU22 and BWCW-SW1 have similar genome characteristics and structures, displaying a 98 % nucleotide sequence identity each to other (Woo et al., 2014).

A novel betacoronavirus distantly related to MERS-CoV was detected in the faeces of European hedgehogs (Erinaceus europeaeus), an insectivorous mammal belonging to a related order of Chiroptera, from Germany. The virus was tentatively referred to as Erinaceus CoV (EriCoV) (Gorman et al., 2014b) and CoVs found in hedgehogs in France, England and Italy had an identity from 92% to 98 % with the EriCoV (Monchatre-Lery et al., 2017; Saldanha et al., 2019; Delogu et al., 2020). These hedgehog CoVs are are now included in a unique species, Hedgehog coronavirus 1 (subgenus Merbecovirus). The virus was not associated to any form of disease, so that Western European hedgehog is a reservoir host of EriCoV in the absence of apparent disease, suggesting that hedgehogs in addition to bats may contribute to the evolution of Merbecovirus (Saldanha et al., 2019). A slightly
divergent *Merbecovirus* was later found in Amur hedgehogs (*Erinaceus amurensis*) in China and was pospomed as a prototype of a separate species, namely *Erinaceus amurensis* hedgehog coronavirus HKU31 (E-HedCoV HKU31) (Lau et al., 2019). A novel coronavirus, named Wénchéng shrew coronavirus (WESV) was detected in shrews (*Suncus murinus*) in China (Wang et al., 2017). WESV is highly divergent from other alphacoronaviruses, exhibiting less than 71.1% amino acid similarity to any known members of the genus *Alphacoronavirus* in the coronavirus-wide conserved domains of the replicase polyprotein pp1ab and less than 61.3% amino acid similarity to the other three coronavirus genera. However, taking into account the current ICTV criteria, WESV is sufficiently divergent to be considered a distinct member of the genus *Alphacoronavirus*, but not a new genus of the subfamily *Orthocoronavirinae* (Wang et al., 2017).

4. Synoptic summary

CoVs have been known in veterinary medicine since many decades; some of these viruses, such as IBV, swine enteric CoVs, BCoV and mus tediled CoVs, can cause diseases that have a great impact on the farm industry. Other CoVs, namely FIPV, FRSCV and MHV, cause severe disease in companion (cats, ferrets) or laboratory (mice) animals. Animal CoVs are paradigmatic on how CoVs evolve through accumulation of point mutations and homologous (and heterologous) recombination, generating different genotypes and pathotypes. These virus variants may have different antigenic properties, escaping the host immunity induced by vaccines, as is the case of IBV. Alternatively, they may have a different tissue tropism in the same host that can increase or decrease the virus pathogenicity, as observed for the virus pairs FECV/FIPV or FREV/FRSVC and TGEV/PRCoV, respectively. In other circumstances, the CoV evolution may result in the switch of the host range from one animal species to another one or from animals to humans. The former event is well documented in veterinary medicine, with a plethora of viruses being originated from IBV and BCoV that adapted to different animal species. However, the most interesting scenario is the jumping and further adaptation of an animal CoV to humans. There is increasing evidence that all HCoVs currently known recognise an animal origin, with bat or rodent CoVs being the most probable ancestors. In most instances, it was suggested that other mammals served as intermediate hosts prior to final adaptation to humans, i.e., alpacas and cattle for the low-pathogenic HCoV-229E and HCoV-OC43, respectively, and wild carnivores and dromedary camels for the high-pathogenic SARS-CoV and MERS-CoV, respectively. Other two HCoVs, namely HCoV-NL63 and HCoV-HKU1, were likely derived from bats and rodents, respectively, but whether this transmission required an intermediate mammalian host is presently unknown. The origin of SARS-CoV-2 should be zoonotic, since highly related sequences were detected in bats, but a definitive intermediate host has been not identified so far. What should we expect from the current pandemic? When HCoV-OC43 crossed the species barrier to infect humans from domestic livestock around 1890, an epidemic of respiratory infection was recorded. Even though, several years later, influenza was suspected to be the cause of it, in that pandemic involvement of central nervous system was more pronounced than in other influenza outbreaks. This evidence is further supported by molecular studies claiming that the most recent common ancestor of BCoV and HCoV-OC43 emerged around 1890 (Vijgen et al., 2005) and by the fact that HCoV-OC43 can be neuroinvasive (Arbour et al., 2000). Likely, HCoV-OC43 crossed species to infect dogs becoming established in this species as CRCoV (Lorusso et al., 2009). A similar scenario could be observed with SARS-CoV-2 with dogs and, at a greater extent, cats. Apparently, cats represent, within the domestic animals which have been experimentally infected, the host, together with ferrets, which is able to sustain more efficiently SARS-CoV-2 replication (Shi et al., 2020). Furthermore, based on structural studies and biochemical experiments, SARS-CoV-2 seems to have an RBD that binds with high affinity to ACE2 also from ferrets and cats (Andersen et al., 2020). Reasonably, a full comprehension of the animal CoV molecular evolution, host range and pathobiology is beneficial to better understand the mechanism driving the emergence and adaptation to humans of zoonotic CoVs.

5. Conclusions

The present review has highlighted that in the last 18 years, also thanks to the availability of novel sequencing technologies, we have witnessed a large number of novel CoVs being discovered in a large number of animals. Truth to be told, it was difficult for us to summarise, in this single review, all CoVs detected in animals and the tight interaction existing between them and human CoVs. Among animals, it is evident that bats are the group of mammals that harbor the largest number of CoVs and that many other animal CoVs recognise their ancestors in bat CoVs.

In an excellent review (Cui et al., 2019) written by the group coordinated by Dr. Zheng-Li Shi of the Wuhan Institute of Virology, Hubei, (China), city infamously known for being the epicenter and origin of the COVID-19 outbreak, authors stated that “...given the prevalence and great genetic diversity of bat SARS-CoVs, their close coexistence and the frequent recombination of CoVs, it is expected that novel variants will emerge in the future”. This forecasting statement was not surprising to coronavirologists and it was not, importantly, surprising to those scientists that daily deal with the plethora of viruses existing at the human/animal health interface. Although scientists were well aware of this hazard, no substantial actions were taken forward the limitations of strict and repeated contacts between humans and wildlife. Indeed, whereas biological mechanisms underlying viral evolution are not under human control, social and cultural habits can be modified accordingly through a deep and pounding informative campaigns. If to the human habits we sum the impact of modern agricultural practices and urbanization and the decrease of vital space for wildlife, it is quite easy to understand that, if countermeasures are not taken, we will face novel serious health emergencies of animal origin in the following years with tremendous social and economic impact on our lives. As clearly demonstrated by the SARS-CoV-2 emergence, CoVs are the main characteristics of this intricate puzzle characterised by the interactions of viral biological mechanisms and human habits.

Our review was reasonably prepared also to highlight (once more!) how CoVs originate, evolve, jump, mutate and infect their host. Could have the current COVID-19 outbreak been avoided? Answering this question is not relevant now, but actions to avoid the next viral spillover from animals to humans is certainly a priority. This task needs to be coupled with massive genomic surveillance in wild animals not limited to CoVs. Massive sequencing of SARS-CoV-2 strains detected in humans and CoVs of wildlife will help further assess the origin of this novel human pandemic and plan future measures able to reduce the risk of emergence of new CoV spillover events. However, additional tasks should be provisionally addressed in order to reduce the risk of future CoV pandemic like the current one. These include: i) prevention of animal-to-human infections through a ban of the wet markets and a more friendly management of the environment; ii) studies on CoV-host interactions to be performed both in *vitro* (cell cultures, ex-vivo explants of the respiratory tract) and *in vivo* (animals susceptible to SARS-CoV-2 infection); iii) development of new antiviral drugs and evaluation of their efficacy in cell cultures and animal models.

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