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Perspective

PERSPECTIVE

Coronaviruses: a paradigm of new emerging zoonotic diseases

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One sentence summary: The global emergency due to the novel type of coronavirus (2019-nCoV) appeared in China highlights the high zoonotic potential of coronaviruses and the need of international collaboration to block viral spread.

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ABSTRACT

A novel type of coronavirus (2019-nCoV) infecting humans appeared in Wuhan, China, at the end of December 2019. Since the identification of the outbreak the infection quickly spread involving in one month more than 31,000 confirmed cases with 638 death. Molecular analysis suggest that 2019-nCoV could be originated from bats after passaging in intermediate hosts, highlighting the high zoonotic potential of coronaviruses.

Keywords: coronavirus; SARS; MERS; 2019-nCoV; zoonosis

CORONAVIRUSES

Coronaviruses (CoVs) are important pathogens for humans and animals, usually associated to respiratory and gastro-intestinal infections. CoVs are spherical enveloped viruses with a diameter of 100–160 nm. Each particle contains a positive-sense single-stranded RNA genome of 27–32 kb that interacts with the nucleoprotein. Viral envelope includes three different proteins: Membrane (M), Envelope (E) and Spike (S) proteins. M protein binds nucleocapsids and is involved in viral assembly and budding; E protein contributes to viral morphogenesis and release as well as to pathogenesis. Finally, S protein forms homotrimeric spikes that recognize the cellular receptor, thus allowing viral entry into target cells (Chen, Liu and Guo 2020).

Coronaviruses affecting human health belong to the family of *Coronaviridae*, subfamily *Coronovirinae*. Among the four genera included in this subfamily, only *Alphacoronavirus* and *Betacoronavirus* are of interest for human and clinical virologists (Table 1).

Until 2002, CoVs were considered minor pathogens for humans, generally associated to common cold or mild respiratory infections in immunocompetent people, with rare exceptions represented by severe infections in infants, young children and elder people (Channappanavar and Perlman 2017). This concept completely changed with the emergence of a highly pathogenic zoonotic disease, the Severe Acute Respiratory Syndrome (SARS) caused by the SARS-CoV. SARS mainly presented as a severe pneumonia, involving at the end more than 8000 patients with a fatality rate of roughly 10% (Kuiken *et al.* 2003; de Wit *et al.* 2016). SARS emerged in 2002 in Guangdong (southern China) and over 2003 its travel-related diffusion in 29 countries/regions represented a global health concern (WHO 2020a). However, due to a strong international response based on quarantine procedures, tracing of contacts and control of potential sources of infection, SARS epidemics was blocked before becoming a pandemic disease. At the beginning of the SARS outbreak, the first patients reported an exposure with animals before the onset of symptoms suggesting a zoonotic origin of this syndrome. Several efforts addressed the identification of the source of the infection, a key step for the control procedures to stop the chain of contagion. Seroepidemiology investigations identified masked palm civets (*Paguma larvata*), sold in a market of live animals, as the possible origin of human infections, as individuals in contact with the same animals were positive for antibodies against SARS-CoV (Cui, Li and Shi 2019). However, studies in the field suggested that masked palm civets

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Table 1. Classification and source of Coronaviruses affecting humans until the emergence of the 2019-nCoV.

Genus	Virus	Natural host	Intermediate host	Disease	Receptor
Alphacoronavirus	HCoV-NL63	Bats	Unknown	Mild respiratory tract infections	Angiotensin converting enzyme 2
	HCoV-229E	Bats	Camelids	Mild respiratory tract infections	Human aminopeptidase N
Betacoronavirus	HCoV-OC43	Rodents	Bovine	Mild respiratory tract infections	9-O-acetylsialic acids
	HCoV-HKU1	Rodents	Unknown	Mild respiratory tract infections and pneumonia	9-O-acetylsialic acids
	SARS-CoV	Bats	Masked palmes civetes	Severe acute respiratory syndrome	Angiotensin converting enzyme 2
	MERS-CoV	Bats	Camelids	Severe acute respiratory syndrome	Dipeptidyl peptidase-4
	2019-nCoV	Bats	Unknown	Severe acute respiratory syndrome	Angiotensin converting enzyme 2

functioned as intermediate hosts between an unknown animal reservoir and humans. In agreement, several CoVs sequences, some of them related to SARS-CoV, were detected in bat species. These findings support the hypothesis that bats represent the main viral reservoir for CoVs and that SARS-CoVs originated from bats. Interestingly, Cui and coworkers highlighted that the gene encoding for the S protein, that determines the viral tropism and host spectrum, shows a high level of variability on the receptor binding domain, an indication of viral adaptation in the different bats species and other animal hosts (Cui, Li and Shi 2019). Of note, among CoVs identified in bats, only one presented a sequence for the S protein that allows the interaction with the angiotensin converting enzyme 2 (ACE2), the human receptor adopted by the SARS-CoV to infect epithelial cells in the lower respiratory tract. This observation suggests that new spillover events, from animal reservoir to humans, require the modification of the S protein to acquire the ability to infect human cells, a key step to overcome the specie barrier. As expected, in addition to S, other viral genes (such as orf8) can mutate during the viral adaptation to the new host, as reported for the SARS-CoV (Cui, Li and Shi 2019). Overall, molecular epidemiology and phylogenetic studies on CoVs highlight the plasticity of their genome. The RNA positive CoVs genome is bigger (≈30 kb) than that of most RNA viruses (≤10 kb). Data indicate that several recombination events have taken place between the genome of different CoVs. Therefore, the ability to originate new viral variants can promote jumps from bats to other sylvatic animals, the adaptation to the new host and then the transmission to domestic animals or humans (Cui, Li and Shi 2019).

A total of 10 years after the onset of SARS outbreak a new coronavirus jumped from animals to humans causing the lethal Middle East Respiratory Syndrome (MERS) (WHO 2020b). The new emerging disease was first identified in humans in 2012 and interested mainly the Kingdom of South Arabia with the majority of reported cases (Zaki *et al.* 2012). In comparison to SARS, MERS is still active and sporadic outbreaks have been reported until now. This respiratory disease is particularly severe, accounting for 2465 cases confirmed by laboratory analyses, with a fatal rate of 35% (de Wit *et al.* 2016; WHO 2020b). Epidemiological studies have suggested that MERS is due to a CoV transmitted by the contact with dromedary camels or camel products (Chan *et al.* 2015). The origin of human infections through the camelids is demonstrated by virus neutralization experiments and by the viral genomic sequence analyses. Interestingly, MERS-CoV- like viruses have been identified in different bat species suggesting that bats functioned as the source of this new human CoV as well. Furthermore it was demonstrated that MERS-CoV can infect bat cells by exploiting the dipeptidyl peptidase-4 (DPP4), the same receptor used to infect human cells (Raj *et al.* 2013). Seroepidemiology tests on samples collected from camels suggested that the passage from bats to camel happened more than 30 years ago and that the virus spread to camelids in Middle East, Asia and Africa (Müller *et al.* 2014; Cui, Li and Shi 2019). However, phylogenetic analyses showed a gap between bat CoVs and the MERS-CoV, suggesting the existence of others CoVs that have not been detected yet (Cui, Li and Shi 2019). Indeed, the number of known CoVs harboured by bats identified in the field studies could increase in the next future.

THE NEW 2019 CORONAVIRUS

At the end of December 2019 the news of an outbreak of a respiratory disease of unknown aetiology in Wuhan city, Hubei province of China, started to circulate around the world (WHO

Table 2. Key events of the 2019-nCoV outbreak.

Date	Event
31 Dec 2019	Wuhan Municipal Health Commission in Wuhan City, China, reports a cluster of 27 pneumonia cases of unknown aetiology,
7 Jan 2020	Identification of the novel coronavirus (2019-nCoV)
9 Jan 2020	China CDC reported that the 2019-nCoV had been detected as the causative agent for 15 of the 59 pneumonia cases
10 Jan 2020	First 2019-nCoV genome sequence was made publicly available
13 Jan 2020	First case of infection outside of China (1 imported case in Thailand)
21 Jan 2020	Chinese Authorities confirms the human-to-human transmission
30 Jan 2020	WHO declares the Public Health Emergency of International Concern

2020c). The origin of the outbreak was linked to Huanan Seafood Wholesale Market. Clinical signs, similar to SARS and MERS, are fever, cough and difficulties in breathing, with an evolution to severe pneumonia in the most severe cases. After few days from the outbreak announcement, Chinese scientists obtained and published genomic sequences of the putative aetiological agent, a novel betacoronavirus, provisionally called 2019-nCoV (Zhou *et al.* 2020; Zhu *et al.* 2020).

Starting from the experience of SARS outbreak management, with the attempt to control the infection, the market was immediately closed and decontaminated the first of January, 2020. Furthermore, with the increasing of human cases, quarantine of infected or potentially infected individual was adopted, and safety guidelines were issued and publicized to the general population to reduce the chances of infection. The picture of an emerging, rapidly evolving situation quickly appeared, with information increasing day by day (Table 2). Unfortunately, the number of cases increased at a high rate, and by the 7th of February 31 503 lab-confirmed cases were confirmed with 638 deaths (ECDC 2020a). The virus moved also outside China and human cases were detected in other Countries of Asia (221), and in different continents, such as Europe (31), America (19) and Oceania (15) (ECDC 2020b). The epidemiological data allowed to estimate a transmission rate of 1.4–2.5, similar to the one observed at the beginning of the SARS outbreak and of the novel influenza virus A(H1N1)pdm09 (Read *et al.* 2020). Clinical information suggested that severe symptoms and death are frequent in elderly, in people with comorbidity, and that infection involve mainly adults (Huang *et al.* 2020; Li *et al.* 2020). Data obtained from familiar outbreaks and confirmed cases reported outside of China supported the human to human transmission (Chan *et al.* 2020; ECDC 2020c). Although it has been suggested that 2019-nCoV can be transmitted by asymptomatic patients (Rothe *et al.* 2020), a clear cut demonstration is still missing. This aspect is crucial for its impact on the efficacy of virus control strategies (Kupferschmidt 2020).

Taking into account the constant increasing of infected individuals and deaths, and the potential pandemic spread of 2019-nCoV around the world, the 30th of January 2020 WHO declared the Public Health Emergency of International Concern (WHO 2020c).

Due to the technological advances of the last decade in genome sequencing, 2019-nCoV genomic sequences were quickly available, allowing the development of specific molecular tests; a step crucial not only for the laboratory confirmation of clinical cases, and thus for drawing a correct epidemiological picture and for spreading containment, but also for the identification of the animal host/s and for environmental screening. It has to be mentioned that epidemiological data strongly suggest the involvement of still untraced sources of transmissions in addition to the seafood market in Wuhan (Nishiura *et al.* 2020). Furthermore, the original source of the infection and the specific association with animal hosts have not been identified yet.

Although a preliminary report based on the codon usage suggested snakes as the possible origin of the novel coronavirus (Ji *et al.* 2020), the current consensus support the hypothesis of the involvement of mammalians or birds. The phylogenetic analysis of the 2019-CoV genome showed that this virus is closely related to the bat CoV RaTG13, thus representing a distinct lineage with respect to SARS and bat SARS-like CoVs (Paraskevis *et al.* 2020; Zhou *et al.* 2020). A combination of modelling approaches and experimental evidence indicate that spikes of the 2019-nCoV recognize the ACE2 receptor, allowing entry into target cells (Letko and Munster 2020; Wan *et al.* 2020; Zhou *et al.* 2020). Overall, data collected so far strongly suggest that the 2019-CoV is a new human-infecting betacoronavirus likely originated from bats jumped to humans probably after the passage in one or more intermediate hosts (Lu *et al.* 2020). Recent data on 2019-nCoV molecular evolution suggest that two different viral strains of 2019-nCoV are involved in the outbreak and that the first passage to humans might have occurred a few months earlier than the identification of the first cases in Wuhan (Xiong *et al.* 2020).

In addition and in parallel to a deep biological characterization, research is currently focused on the crucial aspect of developing effective prophylactic and/or therapeutic interventions to control infection and/or spreading of this novel CoV. So far, treatments adopted are based on previous experience with SARS, MERS or influenza virus. For instance, a combination of interferon-alpha and lopinavir/ritonavir is under evaluation as anti 2019-nCoV therapy. Lopinavir and Ritonavir are known antiretroviral drug targeting protease of the human immunodeficiency virus (HIV). However, CoVs are characterized by a 3C-like protease (3CL) which differs from the aspartyl protease of HIV. As the HIV-1 protease inhibitors are structure-based designed drugs their efficacy on 2019-nCoV has to be proven. Although lopinavir/ritonavir have been adopted for the treatment of SARS, as they lack elements of specificity towards CoV protease, their efficacy is not expected to be sufficient to control viral infection.

Recently, Wang and co-workers reported that Remdesivir, a nucleotide analogue prodrug initially developed for the treatment of Ebola and Marburg virus infection, and the anti-malarial drug chloroquine are effective to inhibit 2019-nCoV replication *in vitro* (Wang *et al.* 2020). Finally, the development and use of neutralizing monoclonal antibodies, as proposed in the case of SARS and MERS, could be envisioned for 2019-nCoV control as well (Kim *et al.* 2019; Goo *et al.* 2020). Many monoclonal

antibodies neutralizing SARS-CoV and MERS-CoV infections have been identified, and some of them displayed a potent inhibitory effect in *in vitro* and *in vivo* experiments. In fact, it has been shown that antibody administration fully protected mice from viral challenges (Jin *et al.* 2017). Thus, as reported for Ebola virus, the use of monoclonal antibodies to treat severe CoVs infections could be a promising strategy (Jin *et al.* 2017).

In conclusion, although scientific programmes to develop vaccines and drugs have been already announced, at the moment, no specific antiviral drugs or vaccines are available for 2019-nCoV and the path from *in vitro* studies to *in vivo* applications of any effective molecule will likely take several months/few years.

FUTURE PERSPECTIVES

After the discovery of SARS-CoV, research studies by several groups have been focused on identifying and characterizing animal CoVs, with the aim to evaluate their zoonotic potential. The high diversity between CoVs detected in bats and the genetic mechanisms to increase their genomic variability increase the risk of interspecies transmission. In agreement, the emergence of the 2019-nCoV highlights the importance of bats as a reservoir for new viruses capable of infecting humans, but also serves as an excellent model to design studies and strategies to prevent future emergence of new zoonotic agents. In this regard, it is important to increase the efforts aimed at the characterization of the virome of different animals to study the viral evolution and adaptation to their natural hosts (Holmes, Rambaut and Andersen 2018). The combination of the new molecular/bioinformatics technologies with the classical virological techniques applied to viral models can provide knowledge to prevent new dangerous spillover events. In particular, the possibility to predict the interspecies transmission, can facilitate the planning of specific surveillance programmes to identify outbreaks as soon as they start. Finally, the establishment of platforms to optimize vaccine development is crucial, as vaccines represent the best measure to avoid spread of infection. In this regard, the possibility to rapidly sequence viral genomes combined with synthetic biology approaches, could speed up the procedures for the identification of vaccine against emerging viruses.

However, it has to be kept in mind that while viral isolation, along with technological advances, might allow the rapid setting up of preventive and therapeutic interventions, their *in vivo* validation and application are still required and are likely time-consuming. Thus, an effective world-wide collaboration in containment measures and more efforts on a global preparedness/response system against emerging infectious diseases are absolutely mandatory.

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