The Human Coronaviruses

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Introduction

Although human coronaviruses (CoV) are known as human pathogens since the 1960s, their virus family has gained notoriety in 2002 and 2003 with the first outbreak of the SARS coronavirus epidemic and with the recent emergence in 2012 of the MERS coronavirus.

Coronaviruses belong to the family Coronaviridae and are enveloped single-stranded RNA viruses with positive RNA-genomes [1]. Their genome is about 26–32 kilobases long and thus represents the longest know viral RNA genome. The name coronaviruses is based on electron microscopy photographs which stimulated the imagination of early electron microscopy analysts who thought that the viruses have a crown-like surface. Consequently, these researchers named the viruses according to the Latin word for crown, i.e., *corona* [2]. Until today, all known coronaviruses share a similar genome organization and expression profile of their genomes: 16 nonstructural proteins (named nsp1–16) are encoded by an open reading frame (ORF) named 1a/1b which is located at the 5′ terminus of the genome, followed by the structural proteins (spike/S, envelope/E, membrane/M, nucleocapsid/N) that in total are encoded by ORFs located 3′ of the viral genome.

Within the family of coronaviruses, four genera exist which are named alpha-CoV (or group 1), beta-CoV (group 2), gamma-CoV (group 3), and delta-CoV (group 4), whereby group 2 coronaviruses comprises four lineages named A, B, C, and D, respectively [2]. In this context it is worth mentioning that the lineage A viruses of the group 2 CoVs encode a smaller protein called hemagglutinin esterase (HE), which appears to be functionally similar to the S protein [3].

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HCoV Genome Organization

As mentioned previously, the human coronaviruses have a non-segmented positive-stranded RNA genome. Approximately 60–70% of this genome consist of two large and overlapping open reading frames (ORF1a and ORF1b) that encode for the polyproteins pp1a and pp1ab that in turn are processed into the 16 nonstructural proteins 1–16. The structural proteins E, M, N, and S share the rest of the ORFs of the viral genome while being accompanied by a variable number of the so-called accessory proteins [2]. The long genomes are believed to originate from a unique replication fidelity that in turn is originated by a set of viral enzymes harboring RNA-processing functions [4].

Clinical Symptoms

In humans, HCoV infections in general result in self-limiting disease courses that involve the upper respiratory and the gastrointestinal tract. Symptoms may vary from mild to serious and (sometimes) life-threatening infections in permissive patients and range from a common cold to bronchitis and pneumonia; occasionally renal involvement is seen [5–15].

In this context it is important to note that the clinical manifestations of the two most serious (but also least frequent) HCoVs, namely, SARS coronavirus and MERS coronaviruses, are more serious and frequently are life-threatening. However, despite the ongoing endemic MERS outbreak in the Arabian region and single outbreaks in South Korea, these two pathogens remain limited to single outbreaks (in case of SARS-CoV) and endemic zoonotic transmissions in the Middle East area.

In any case, none of the remaining human coronaviruses can be identified on clinical symptoms alone, and coinfections with other respiratory viruses are as common as with other respiratory pathogens, making it difficult to identify which is the "leading" pathogen in multiple infections [16–22].

Epidemiology

To date, six human coronaviruses have been discovered, i.e., the human coronaviruses OC43 and 229E, NL63 and HKU1, and the SARS and MERS coronaviruses. Except for the latter two, all human coronaviruses have been noted to occur worldwide and are mostly associated with a seasonality that follows the typical flu-like symptom season [23–31]. As the nomenclature of coronaviruses is far from being logical, these viruses are described in the next section in more detail according to their systematic order.

Human Coronavirus 229E (Group 1/Alpha-Coronavirus)

Occurring globally, the human coronavirus type 229E was initially discovered in 1966 during a trial to identify several newly recognized pathogens associated with the common cold [32, 33]. The clinical symptoms associated with 229E include malaise, headache, sneezing, sore throat, sometimes fever, and cough. The time span between infection and clinical symptoms is reported between 2 and 5 days with clinical symptoms lasting between 2 and 18 days [34–37]. Anyway, as mentioned earlier, there is no clinical difference between 229E infections and other respiratory infections caused by viral pathogens such as rhinovirus or influenza A [34–37].

Recently it has been postulated that 229E originated from a recombination event between the alpaca alpha-coronavirus. This recombination event occurred within the S gene and was followed by a deletion in the same gene [38].

Human Coronavirus NL63 (Group 1/Alpha-Coronavirus)

Discovered in 2004, the human coronavirus NL63 has been found worldwide since then and is mainly associated with respiratory infections in children, the elderly, and immunocompromised patients. The virus was consecutively discovered in two separate laboratories in the Netherlands, one in Amsterdam and one in Rotterdam [39, 40]. NL63 infections in general present with mild respiratory symptoms such as cough, rhinorrhea, tachypnea, fever, and hypoxia [11, 13, 41–44] and are self-limited. A frequently observed "complication" is croup which is present in approx. 5% of NL63 infections [45].

Human Coronavirus HKU1 (Group 2/Betacoronavirus, Lineage A)

Starting with the description of the human metapneumovirus in 2001, a new era in virology began; this era focused on viral discovery methods that combined classical techniques of virology with modern molecular methods. The resulting wave of virus discoveries led to another trend in molecular diagnostics in which singleplex step by step methods were replaced with multiplexing technologies able to screen for several pathogens simultaneously. During this time, HKU1 was detected in 2005 at the **H**ong **K**ong **U**niversity (which is also the institution from which the name HKU1 was derived). The isolation of HKU1 was from an elderly patient who suffered from bronchiolitis and pneumonia [46–48]. Fatal infections occur rarely, and the infections are indistinguishable from other viral respiratory infections. As the other "common cold" coronaviruses, HKU is circulating globally [49–54].

Human Coronavirus OC43 (Group 2/Betacoronavirus of Lineage A)

The strain OC43 belongs to the longest known human coronaviruses and was identified in 1967 [55, 56]. The discrimination between OC43 and 229 can be performed exclusively by molecular methods or serologically, and both viruses have the same morphology and clinical spectrum [55, 56].

SARS Coronavirus (Group 2 Coronavirus/Betacoronavirus of Lineage B)

Much has been speculated; even more has been confirmed about the SARS coronavirus since it was first detected in 2002/2003 during an outbreak in China. The subsequent pandemic that was beginning was halter due to strict hygienic procedures and intervention measures before a worldwide disaster could occur. As a matter of fact, the discovery of this virus was possible solely by the first alarming observations reported by Dr. Carlo Urbani [57], a physician who was confronted with patients suffering from fever, myalgia, headache, malaise, and chills followed by a dry cough, dyspnea, and respiratory distress; in some cases infections of the liver, kidney, gastrointestinal tract, and brain occurred [58–62]. The overall mortality rate is 9% but is higher with increasing age. To date, the SARS coronavirus has caused only a single outbreak followed by spread to other locations as a result of travel. This initial SARS coronavirus outbreak is now known to be an archetypic zoonosis outbreak of this virus or other SARS-like coronaviruses. Such coronaviruses circulating in their natural reservoirs should not be excluded during and outbreak and require a narrow mesh of surveillance.

MERS Coronavirus (Group 2/Betacoronavirus, Lineage C)

The MERS coronavirus first came to the attention of the scientific community in 2012 when the virus was isolated for the first time in Saudi Arabia. It causes severe pneumonia with acute respiratory distress (ARDS) and is frequently associated with gastrointestinal symptoms. Importantly, renal impairment is frequently observed. Especially patients with an underlying comorbidity are permissive for MERS-CoV infections and have a high mortality rate [63–75]. It is important to note that, although the virus appears to be endemic, spontaneous outbreaks due to imported cases are possible, as most recently reported from South Korea, where the roommate of an index patient left the hospital on his own account and thereby caused a

local outbreak [76–79]. It is worth noting that in terms of the MERS-CoV, it is assumed that the viral spike protein enables the virus to evade the immune system by preventing the binding of neutralizing antibodies.

Virus Ecology of Human Coronaviruses

To date it appears that the coronaviruses NL63, HKU1, 229E, and OC43 are well-adapted human viruses that remain in the human reservoir; these coronaviruses originated from zoonotic transmission long ago [38, 80–83]. In contrast, MERS-CoV and SARS-CoV are less adapted to the human host and most likely represent zoonoses, originating from their natural reservoirs camels and bats, respectively [82–90].

Diagnostics

The diagnostic confirmation of a human coronavirus infection does not necessarily lead to a specific therapeutic decision. While coronaviruses NL63, HKU1, OC43, and 229E do not require "special" attention, isolation of patients is strictly required in case of SARS-CoV and should be considered in case of MERS-CoV.

As diagnostic methods, neither cell culture-based nor electron microscopy methods are the first choice. Instead, molecular methods such as RT-qPCR, LAMP, or multiplexing methods should be used. RT-qPCR protocols have been described by several groups and are the method of choice for the new coronaviruses. For MERS coronavirus it is recommended by Corman and coworkers to use the upE region and the Orf1a as targets for the PCR, while Orf1b has a reduced sensitivity [91]. In addition, it is recommended to sequence parts of the RdRp- and/or the N-gene to confirm the results. Internal and external controls should be included in every PCR run and are available, e.g., from Public Health England.

For the other coronaviruses, several validated and approved multiplex assays are available, such as the RespiFinder assay (Pathofinder, Maastricht, Netherlands), the film array (former IDAHO film assay, meanwhile produced and distributed by bioMerieux, Lyon, France), or the Luminex RVP (Luminex, Austin, Texas, USA). All of these assays have the advantage of a high sensitivity combined with the simultaneous detection of several other pathogens. Moreover, the novel Light Mix Modular Assays from Roche/TIBMOLBIOL could serve as an alternative for coronavirus diagnostics.

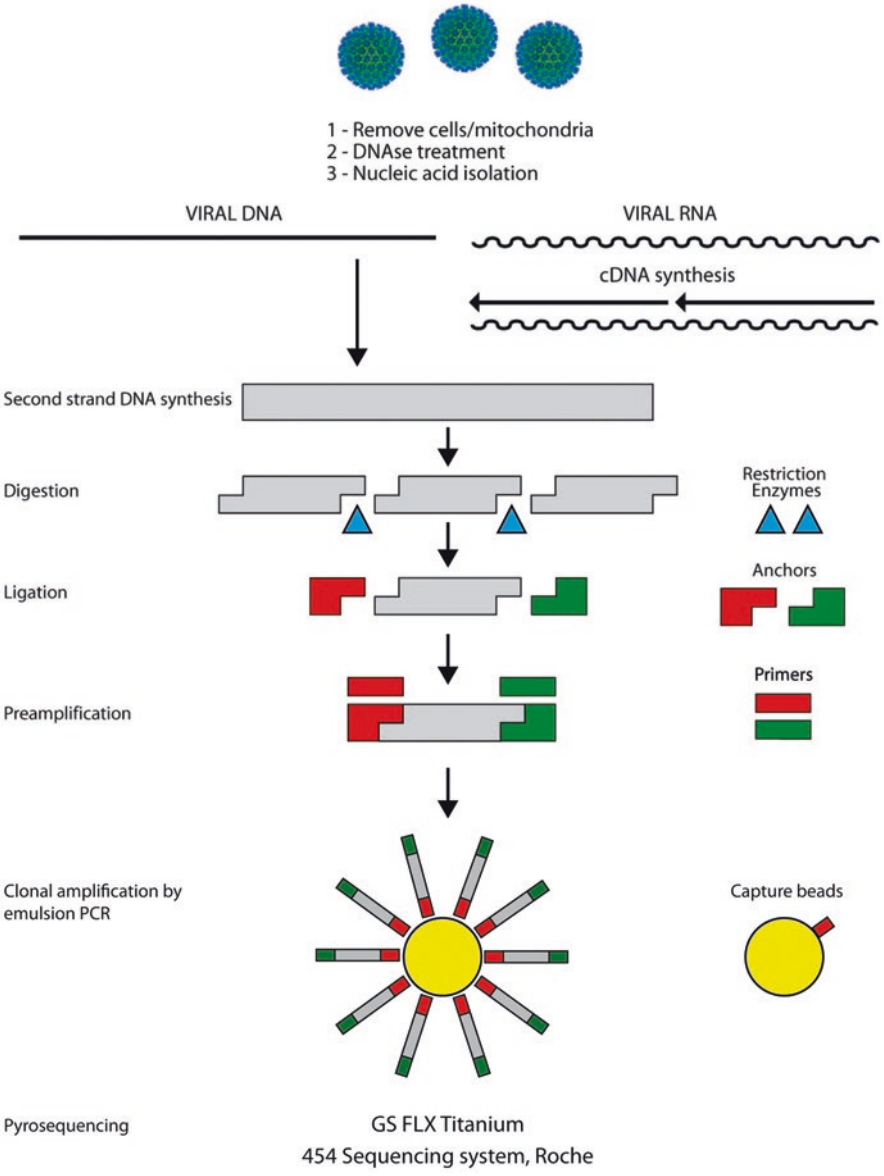

Fig. 1 Overview of the novel high-throughput VIDISCA method. (From de Vries et al. 2011, PLoS One [92]. Original picture published under the Creative Commons Attribution (CC BY) license in PLoS One [92])

Advanced Molecular Techniques Relevant to Human Coronaviruses

The detection of novel coronaviruses within the last 15 years are excellent examples for the necessity of advanced molecular techniques that have to be combined with classical virological methods. As an example, the discovery of the SARS coronavirus has become possible solely due to the sophisticated combination of detailed and timely clinical observation followed by attempts to isolate the virus in cell culture (classical method) and subsequent characterization by modern molecular techniques. The latter method used for the identification of the novel genome of the SARS coronavirus was called random reverse transcriptase PCR and led to the amplification and subsequent sequencing of the first known SARS genomes [62].

A further example is the discovery of the human coronavirus NL63 by van der Hoek and coworkers [39]. These researchers established a novel method called VIDISCA (virus discovery cDNA-AFLP). For this method, the viral DNA or cDNA is digested with enzymes targeting short recognition sequences that are virtually present in all viruses. These fragments are then ligated to adaptors and amplified by an adaptor-specific PCR. The VIDISCA method meanwhile was refined (Fig. 1) and is applicable as a sensitive assay for virus discovery also from clinical samples [92].

Concluding Remarks

Coronaviruses have been recognized as a major player in serious airway infections. The recent experiences with the MERS coronavirus and the outbreak experience with the SARS coronavirus have shown that these zoonotic viruses are able to cross the species barrier and along with influenza viruses are the most likely candidates for future outbreaks. In concert with newer studies on virus ecology, it has become obvious that coronaviruses are ubiquitous pathogens infecting a broad range of mammals that often are in contact with humans, thus providing the basics for future zoonotic outbreaks.

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