The Human Coronaviruses

Oliver Schildgen

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 known 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].

O. Schildgen
Kliniken der Stadt Köln gGmbH, Institut für Pathologie, Klinikum der Privaten Universität Witten/Herdecke mit Sitz in Köln, Cologne/Köln, Germany
e-mail: schildgeno@kliniken-koeln.de; oliver.schildgen@uni-wh.de
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 Hong Kong University (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 halted 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.

References

1. Lambert S, Mackay IM, Sloots TP, Nissen MD. Human coronavirus nomenclature. Pediatr Infect Dis J. 2006;25(7):662.
2. Masters PS, Perlman S. Coronaviridae. In: Knipe DM, Howley PM, editors. Fields virology. Philadelphia: Wolters Kluwer/Lippincott Williams & Wilkins; 2013. p. 825–58.
3. Langereis MA, Van Vliet AL, Boot W, De Groot RJ. Attachment of mouse hepatitis virus to O-acetylated sialic acid is mediated by hemagglutinin-esterase and not by the spike protein. J Virol. 2010;84(17):8970–4.
4. Lauber C, Goeman JJ, Parquet Mdel C, et al. The footprint of genome architecture in the largest genome expansion in RNA viruses. PLoS Pathog. 2013;9(7):e1003500.

5. Riski H, Hovi T. Coronavirus infections of man associated with diseases other than the common cold. J Med Virol. 1980;6(3):259–65.

6. Falsey AR, Mccann RM, Hall WJ, et al. The “common cold” in frail older persons: impact of rhinovirus and in a senior daycare center. J Am Geriatr Soc. 1997;45(6):706–11.

7. Nokso-Koivisto J, Kinnari TJ, Lindahl P, Hovi T, Pitkaranta A. Human picornavirus and coronavirus RNA in nasopharynx of children without concurrent respiratory symptoms. J Med Virol. 2002;66(3):417–20.

8. Li L, Wang Z, Lu Y, et al. Severe acute respiratory syndrome-associated coronavirus genotype and its characterization. Chin Med J. 2003;116(9):1288–92.

9. Peiris JS, Lai ST, Poon LL, et al. Coronavirus as a possible cause of severe acute respiratory syndrome. Lancet. 2003;361(9366):1319–25.

10. Boivin G, Baz M, Cote S, et al. Infections by human coronavirus-NL in hospitalized children. Pediatr Infect Dis J. 2005;24(12):1045–8.

11. Al-Hameed F, Wahla AS, Siddiqui S, et al. Characteristics and outcomes of Middle East respiratory syndrome coronavirus patients admitted to an intensive care unit in Jeddah, Saudi Arabia. J Intensive Care Med. 2016;31(5):344–8.

12. Juan JL, Chen TC, Jiang SS, et al. Coupling multiplex RT-PCR to a gene chip assay for sensitive and semiquantitative detection of severe acute respiratory syndrome-coronavirus. Lab Invest (A Journal of Technical Methods and Pathology). 2004;84(9):1085–91.

13. Asano KM, De Souza SP, De Barros IN, et al. Multiplex semi-nested RT-PCR with exogenous internal control for simultaneous detection of bovine coronavirus and group A rotavirus. J Virol Methods. 2010;169(2):375–9.

14. Costa E, Rodriguez-Dominguez M, Clari MA, Gimenez E, Galan JC, Navarro D. Comparison of the performance of 2 commercial multiplex PCR platforms for detection of respiratory viruses in upper and lower tract respiratory specimens. Diagn Microbiol Infect Dis. 2015;82(1):40–3.

15. De Vos N, Vankeerberghen A, Vaeyens F, Van Vaerenbergh K, Boel A, De Beenhouwer H. Simultaneous detection of human bocavirus and adenovirus by multiplex real-time PCR in a Belgian paediatric population. Eur J Clin Microbiol Infect Dis. 2009;28(11):1305–10.

16. Huguenin A, Moutte L, Renois F, et al. Broad respiratory virus detection in infants hospitalised for bronchiolitis by use of a multiplex RT-PCR DNA microarray system. J Med Virol. 2012;84(6):979–85.

17. Lassaumiere R, Kresfelder T, Venter M. A novel multiplex real-time RT-PCR assay with FRET hybridization probes for the detection and quantification of 13 respiratory viruses. J Virol Methods. 2010;165(2):254–60.

18. Leung TF, Li CY, Lam WY, et al. Epidemiology and clinical presentations of human coronavirus NL63 infections in Hong Kong children. J Clin Microbiol. 2009;47(11):3486–92.

19. Gerna G, Percivalle E, Sarasini A, et al. Human respiratory coronavirus HKU1 versus other coronavirus infections in Italian hospitalised patients. J Clin Virol (The Official Publication of the Pan American Society for Clinical Virology). 2007;38(3):244–50.
25. Lambert SB, Allen KM, Druce JD, et al. Community epidemiology of human metapneumovirus, human coronavirus NL63, and other respiratory viruses in healthy preschool-aged children using parent-collected specimens. Pediatrics. 2007;120(4):e929–37.

26. Gorse GJ, O’connor TZ, Hall SL, Vitale JN, Nichol KL. Human coronavirus and acute respiratory illness in older adults with chronic obstructive pulmonary disease. J Infect Dis. 2009;199(6):847–57.

27. Al Hajjar S, Al Thawadi S, Al Seraihi A, Al Muhsen S, Imambaccus H. Human metapneumovirus and human coronavirus infection and pathogenicity in Saudi children hospitalized with acute respiratory illness. Ann Saudi Med. 2011;31(5):523–7.

28. Lee WJ, Chung YS, Yoon HS, Kang C, Kim K. Prevalence and molecular epidemiology of human coronavirus HKU1 in patients with acute respiratory illness. J Med Virol. 2013;85(2):309–14.

29. Razuri H, Malecki M, Timoco Y, et al. Human coronavirus-associated influenza-like illness in the community setting in Peru. Am J Trop Med Hyg. 2015;93(5):1038–40.

30. Abdulhaq AA, Basode VK, Hashem AM, et al. Patterns of human respiratory viruses and lack of MERS-coronavirus in patients with acute upper respiratory tract infections in Southwestern Province of Saudi Arabia. Adv Virol. 2017;2017(4247853)

31. Kim KY, Han SY, Kim HS, Cheong HM, Kim SS, Kim DS. Human coronavirus in the 2014 winter season as a cause of lower respiratory tract infection. Yonsei Med J. 2017;58(1):174–9.

32. Hamre D, Connelly AP Jr, Procknow JJ. Virologic studies of acute respiratory disease in young adults. IV. Virus isolations during four years of surveillance. Am J Epidemiol. 1966;83(2):238–49.

33. Hamre D, Procknow JJ. A new virus isolated from the human respiratory tract. Proc Soc Exp Biol Med (Society for Experimental Biology and Medicine). 1966;121(1):190–3.

34. Vabret A, Mourez T, Gouarin S, Petitjean J, Freymuth F. An outbreak of coronavirus OC43 respiratory infection in Normandy, France. Clin Infect Dis (An Official Publication of the Infectious Diseases Society of America). 2003;36(8):985–9.

35. Chiu SS, Chan KH, Chu KW, et al. Human coronavirus NL63 infection and other coronavirus infections in children hospitalized with acute respiratory disease in Hong Kong, China. Clin infect Dis (An Official Publication of the Infectious Diseases Society of America). 2005;40(12):1721–9.

36. Principi N, Bosis S, Esposito S. Effects of coronavirus infections in children. Emerg Infect Dis. 2010;16(2):183–8.

37. Ogimi C, Waghmare AA, Kuypers JM, et al. Clinical significance of human coronavirus in Bronchoalveolar Lavage samples from hematopoietic cell transplantation recipients and patients with Hematologic Malignancies. Clin Infect Dis (An Official Publication of the Infectious Diseases Society of America). 2017. https://doi.org/10.1093/cid/cix160.

38. Corman VM, Eckerle I, Memish ZA, et al. Link of a ubiquitous human coronavirus to dromedary camels. Proc Natl Acad Sci U S A. 2016;113(35):9864–9.

39. Van Der Hoek L, Pyrc K, Jebbink MF, et al. Identification of a new human coronavirus. Nat Med. 2004;10(4):368–73.

40. Fouchier RA, Hartwig NG, Bestebroer TM, et al. A previously undescribed coronavirus associated with respiratory disease in humans. Proc Natl Acad Sci U S A. 2004;101(16):6212–6.

41. Arden KE, Nissen MD, Sloots TP, Mackay IM. New human coronavirus, HCoV-NL63, associated with severe lower respiratory tract disease in Australia. J Med Virol. 2005;75(3):455–62.

42. Ebihara T, Endo R, Ma X, Ishiguro N, Kikuta H. Detection of human coronavirus NL63 in young children with bronchiolitis. J Med Virol. 2005;75(3):463–5.

43. Smuts H, Workman L, Zar HJ. Role of human metapneumovirus, human coronavirus NL63 and human bocavirus in infants and young children with acute wheezing. J Med Virol. 2008;80(5):906–12.

44. Wu PS, Chang LY, Berkhout B, et al. Clinical manifestations of human coronavirus NL63 infection in children in Taiwan. Eur J Pediatr. 2008;167(1):75–80.

45. Van Der Hoek L, Sure K, Ihorst G, et al. Croup is associated with the novel coronavirus NL63. PLoS Med. 2005;2(8):e240.
46. Woo PC, Lau SK, Chu CM, et al. Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. J Virol. 2005;79(2):884–95.
47. Woo PC, Lau SK, Huang Y, Tsui HW, Chan KH, Yuen KY. Phylogenetic and recombination analysis of coronavirus HKU1, a novel coronavirus from patients with pneumonia. Arch Virol. 2005;150(11):2299–311.
48. Woo PC, Lau SK, Tsai HW, et al. Clinical and molecular epidemiological features of coronavirus HKU1-associated community-acquired pneumonia. J Infect Dis. 2005;192(11):1898–907.
49. Esper F, Weibel C, Ferguson D, Landry ML, Kahn JS. Coronavirus HKU1 infection in the United States. Emerg Infect Dis. 2006;12(5):775–9.
50. Lau SK, Woo PC, Yip CC, et al. Coronavirus HKU1 and other coronavirus infections in Hong Kong. J Clin Microbiol. 2006;44(6):2063–71.
51. Sloots TP, Mceerlean P, Speicher DJ, Arden KE, Nissen MD, Mackay IM. Evidence of human coronavirus HKU1 and human bocavirus in Australian children. J Clin Virol (The Official Publication of the Pan American Society for Clinical Virology). 2006;35(1):99–102.
52. Woo PC, Lau SK, Yip CC, Huang Y, Yuen KY. More and more coronaviruses: human coronavirus HKU1. Viruses. 2009;1(1):57–71.
53. Pyrc K, Sims AC, Dijkman R, et al. Culturing the unculturable: human coronavirus HKU1 infects, replicates, and produces progeny virions in human ciliated airway epithelial cell cultures. J Virol. 2010;84(21):11255–63.
54. Amini R, Jahanshiri F, Amini Y, Sekawi Z, Jalilian FA. Detection of human coronavirus strain HKU1 in a 2 years old girl with asthma exacerbation caused by acute pharyngitis. Virol J. 2012;9:142.
55. McIntosh K, Becker WB, Chanock RM. Growth in suckling-mouse brain of “IBV-like” viruses from patients with upper respiratory tract disease. Proc Natl Acad Sci U S A. 1967;58(6):2268–73.
56. McIntosh K, Dees JH, Becker WB, Kapikian AZ, Chanock RM. Recovery in tracheal organ cultures of novel viruses from patients with respiratory disease. Proc Natl Acad Sci U S A. 1967;57(4):933–40.
57. Reilley B, Van Herp M, Sermand D, Dentico N. SARS and Carlo Urbani. N Engl J Med. 2003;348(20):1951–2.
58. Chan HL, Tsui SK, Sung JJ. Coronavirus in severe acute respiratory syndrome (SARS). Trends Mol Med. 2003;9(8):323–5.
59. Ding Y, He L, Zhang Q, et al. Organ distribution of severe acute respiratory syndrome (SARS) associated coronavirus (SARS-CoV) in SARS patients: implications for pathogenesis and virus transmission pathways. J Pathol. 2004;203(2):622–30.
60. Lu H, Zhao Y, Zhang J, et al. Date of origin of the SARS coronavirus strains. BMC Infect Dis. 2004;4:3.
61. Kuiken T, Fouchier RA, Schutten M, et al. Newly discovered coronavirus as the primary cause of severe acute respiratory syndrome. Lancet. 2003;362(9380):263–70.
62. Drosten C, Gunther S, Preiser W, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N Engl J Med. 2003;348(20):1967–76.
63. WHO Statement on the third meeting of the IHR Emergency committee concerning Middle East respiratory syndrome coronavirus (MERS-CoV). Releve Epidemiologique Hebdomadaire. 2013;88(40):435–436.
64. MERS coronavirus has low pandemic potential, so far. BMJ 347 f4371; 2013.
65. Bennet N. Alarm bells over MERS coronavirus. Lancet Infect Dis. 2013;13(7):573–4.
66. Cauchemez S, Van Kerkhove MD, Riley S, Donnelly CA, Fraser C, Ferguson NM. Transmission scenarios for Middle East Respiratory Syndrome Coronavirus (MERS-CoV) and how to tell them apart. Euro surveill (Bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin). 2013;18(24)
67. Centers for Disease C. Prevention. Updated information on the epidemiology of Middle East respiratory syndrome coronavirus (MERS-CoV) infection and guidance for the public, clinicians, and public health authorities, 2012–2013. MMWR Morb Mortal Wkly Rep. 2013;62(38):793–6.
68. Centers for Disease C, Prevention. Update: Recommendations for Middle East respiratory syndrome coronavirus (MERS-CoV). MMWR Morb Mortal Wkly Rep. 2013;62(27):557.
69. Centers for Disease C, Prevention. Update: Severe respiratory illness associated with Middle East Respiratory Syndrome Coronavirus (MERS-CoV)–worldwide, 2012–2013. MMWR Morb Mortal Wkly Rep. 2013;62(23):480–3.
70. De Groot RJ, Baker SC, Baric RS, et al. Middle East respiratory syndrome coronavirus (MERS-CoV): announcement of the Coronavirus Study Group. J Virol. 2013;87(14):7790–2.
71. De Wilde AH, Raj VS, Oudshoorn D, et al. MERS-coronavirus replication induces severe in vitro cytopathology and is strongly inhibited by cyclosporin A or interferon-alpha treatment. J Gen Virol. 2013;94(Pt 8):1749–60.
72. De Wit E, Prescott J, Baseler L, et al. The Middle East respiratory syndrome coronavirus (MERS-CoV) does not replicate in Syrian hamsters. PLoS One. 2013;8(7):e69127.
73. De Wit E, Rasmussen AL, Falzarano D, et al. Middle East respiratory syndrome coronavirus (MERS-CoV) causes transient lower respiratory tract infection in rhesus macaques. Proc Natl Acad Sci U S A. 2013;110(41):16598–603.
74. Devitt E. Lack of small animal model hinders MERS coronavirus research. Nat Med. 2013;19(8):952.
75. Eckerle I, Muller MA, Kallies S, Gotthardt DN, Drosten C. In-vitro renal epithelial cell infection reveals a viral kidney tropism as a potential mechanism for acute renal failure during Middle East Respiratory Syndrome (MERS) coronavirus infection. Virol J. 2013;10:359.
76. Hsieh YH. Middle East Respiratory Syndrome Coronavirus (MERS-CoV) nosocomial outbreak in South Korea: insights from modeling. Peer J. 2015;3:e1505.
77. Kim TH, Lee HH. Considerations left behind Middle East respiratory syndrome coronavirus (MERS-CoV) outbreaks in Republic of Korea. J Menopausal Med. 2015;21(2):63–4.
78. Park WB, Perera RA, Choe PG, et al. Kinetics of serologic responses to MERS coronavirus infection in humans, South Korea. Emerg Infect Dis. 2015;21(12):2186–9.
79. Kim KH, Tandi TE, Choi JW, Moon JM, Kim MS. Middle East respiratory syndrome coronavirus (MERS-CoV) outbreak in South Korea, 2015: epidemiology, characteristics and public health implications. J Hosp Infect. 2017;95(2):207–13.
80. Vigen L, Keyaerts E, Moes E, et al. Complete genomic sequence of human coronavirus OC43: molecular clock analysis suggests a relatively recent zoonotic coronavirus transmission event. J Virol. 2005;79(3):1595–604.
81. Huynh J, Li S, Yount B, et al. Evidence supporting a zoonotic origin of human coronavirus strain NL63. J Virol. 2012;86(23):12816–25.
82. Chan JF, Lau SK, To KK, Cheng VC, Woo PC, Yuen KY. Middle East respiratory syndrome coronavirus: another zoonotic betacoronavirus causing SARS-like disease. Clin Microbiol Rev. 2015;28(2):465–522.
83. Gossner C, Danielson N, Gervelmeyer A, et al. Human-dromedary camel interactions and the risk of acquiring zoonotic Middle East respiratory syndrome coronavirus infection. Zoonoses Public Health. 2016;63(1):1–9.
84. Han HJ, Yu H, Yu XJ. Evidence for zoonotic origins of Middle East respiratory syndrome coronavirus. J Gen Virol. 2016;97(2):274–80.
85. Hon CC, Lam TY, Shi ZL, et al. Evidence of the recombinant origin of a bat severe acute respiratory syndrome (SARS)-like coronavirus and its implications on the direct ancestor of SARS coronavirus. J Virol. 2008;82(4):1819–26.
86. Gouilh MA, Puechmaille SJ, Gonzalez JP, Teeling E, Kittayapong P, Manuguerra JC. SARS-coronavirus ancestor's foot-prints in South-East Asian bat colonies and the refuge theory. Infect Genet Evol (Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases). 2011;11(7):1690–702.
87. Balboni A, Battilani M, Prosperi S. The SARS-like coronaviruses: the role of bats and evolutionary relationships with SARS coronavirus. New Microbiol. 2012;35(1):1–16.
88. Lu G, Liu D. SARS-like virus in the Middle East: a truly bat-related coronavirus causing human diseases. Protein Cell. 2012;3(11):803–5.
89. Wang M, Hu Z. Bats as animal reservoirs for the SARS coronavirus: hypothesis proved after 10 years of virus hunting. Virol Sin. 2013;28(6):315–7.

90. Ng OW, Tan YJ. Understanding bat SARS-like coronaviruses for the preparation of future coronavirus outbreaks - implications for coronavirus vaccine development. Hum Vaccin Immunother. 2017;13(1):186–9.

91. Corman VM, Eckerle I, Bleicker T, et al. Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction. Euro Surveill (Bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin). 2012;17(39)

92. De Vries M, Deijs M, Canuti M, et al. A sensitive assay for virus discovery in respiratory clinical samples. PLoS One. 2011;6(1):e16118.