Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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In the two preceding chapters, picornaviruses and flaviviruses were covered. Besides these two positive-strand RNA viruses, caliciviruses, togaviruses, and coronaviruses are also clinically important human pathogens. Here, these positive-strand RNA viruses will be covered only briefly with an emphasis on classification and the genome features.

13.1 CALICIVIRUS

Caliciviruses\(^1\) (family Caliciviridae) are similar to picornaviruses in many respects. Caliciviruses are small (35–40 nm), nonenveloped, and icosahedral viruses that possess a positive-strand RNA genome of 7–8 kb. Caliciviruses are important human and veterinary pathogens which are associated with a broad spectrum of diseases in their hosts. Here, norovirus, a prototype of calicivirus and the causative agent of nonbacterial gastroenteritis in humans, will be covered.

Classification: Caliciviruses comprise four genera (Table 13.1). Norwalk virus, a member of the genus Norovirus, causes epidemic gastroenteritis. Sapporovirus, a member of the genus Sapovirus, also causes epidemic gastroenteritis. Besides human caliciviruses, two veterinary caliciviruses are found. Rabbit hemorrhagic disease virus (RHDV), a member of the genus Lagovirus, is associated with a fatal liver disease in rabbits. Feline calicivirus (FCV), a member of the genus Vesivirus, causes respiratory disease in cats.

13.1.1 Norovirus

Norovirus\(^2\) represents a prototype of calicivirus. Noroviruses are the causative agents of nonbacterial gastroenteritis in humans and are responsible for almost all viral gastroenteritis outbreaks worldwide.

Epidemiology: Noroviruses are transmitted via the oral-fecal route due to contaminated water and food, or directly from person to person. They are extremely contagious. Transmission can be aerosolized when those stricken with the illness vomit, or when a toilet flushes with vomit or diarrhea in it; infection can occur by eating food or breathing air near an episode of vomiting, even if cleaned up. The viruses continue to be shed after symptoms have subsided and shedding can still be detected many weeks after infection.

Noroviruses are responsible for over 50% of gastroenteritis worldwide (Fig. 13.1). Each year, human noroviruses cause at least around 267 million episodes and over 200,000 deaths in developing nations as well as approximately 900,000 cases of pediatric gastroenteritis in industrialized nations. Even in the United States, they cause over 20,000 episodes and 300 deaths. Shellfishes (oysters in particular), which are often consumed uncooked, are frequently the source of norovirus outbreaks.

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1. **Calicivirus** The term is derived from Greek word for “cup”-calyx.
2. **Norovirus** The norovirus was originally named the “Norwalk agent” after Norwalk, Ohio, in the United States, where an outbreak of acute gastroenteritis occurred among children at the elementary school in 1968. The virus was given the name “Norwalk virus.”
source of infection. Oysters act as a filter to concentrate the virus particles present in sea water. Symptoms include vomiting, diarrhea, and dehydration. No vaccine or antiviral drugs are yet available.

**Genome Structure** The RNA genome of norovirus is fundamentally similar to that of picornaviruses, except that a subgenomic RNA is expressed (Fig. 13.2). VPg (virion protein genome-linked) is covalently linked to both the genomic and the subgenomic RNAs. The ORF1 polyprotein is later processed to form six individual proteins by a virus-encoded 3CL\textsuperscript{PRO} protease. RdRp, RNA-dependent RNA polymerase.

**TABLE 13.1 Classification of Calicivirus**

| Genus/Species                        | Acronym | Host            | Transmission | Disease               |
|--------------------------------------|---------|-----------------|--------------|-----------------------|
| Norovirus/Norwalk virus              | NoV     | Humans          | Fecal-oral   | Epidemic gastroenteritis |
| Sapovirus/Sapporovirus               | SV      | Humans, pigs    | Fecal-oral   | Epidemic gastroenteritis |
| Vesivirus/Feline calicivirus         | FCV     | Cats            | Contact      | Respiratory disease    |
| Lagovirus/Rabbit hemorrhagic disease virus | RHDV    | Rabbits         | Fecal-oral   | Hemorrhages            |

**FIGURE 13.1 Norovirus particle.** (Left) A cross section of norovirus capsid particle of 38 nm in diameter. VPg is linked to 5' terminus of the positive-strand RNA genome. The capsid is an icosahedral particles having \( T = 3 \) symmetry. (Right) The electron microscopic image of norovirus particles.

**FIGURE 13.2 RNA genome structure of norovirus.** In addition to the genomic RNA, norovirus has a subgenomic RNA. The VPg is covalently linked to the 5' termini of both the genomic and the subgenomic RNAs. The ORFI polyprotein is later processed to form six individual proteins by a virus-encoded 3CL\textsuperscript{PRO} protease. RdRp, RNA-dependent RNA polymerase.

3. **Coronavirus** The name “coronavirus” is derived from the Latin corona, meaning crown or halo.
founded in the 5' NCR. Instead, the VPg recruits eIF4E, a cap-binding translation initiation factor, to initiate translation (Box 13.1). In other words, the VPg substitutes for the cap structure in recruiting host translation factors.

**Cultivation in Cell Culture:** The investigation on human norovirus has been hampered by the lack of cell lines that support the virus infection. Since human norovirus has remained uncultivable, the studies on viral genome replication could be performed only by transfection of viral genomic RNA into appropriate cells. Recently, major progress in norovirus research was made by a successful demonstration of a human norovirus infection in a cell culture (see Journal Club). Successful establishment of an in vitro cultivation system of human norovirus will facilitate the development of prophylactic vaccine and antivirals.

**Animal Model:** Animal model for human norovirus infection is not yet available. Murine norovirus was recently discovered and will be explored as an animal model for human norovirus.

### 13.2 TOGAVIRUS

Togaviruses (family Togaviridae) are enveloped and icosahedral viruses that possess a positive-sense single-strand RNA genome of 11 kb. Members of this family are frequently referred to as alphaviruses, a genus of this family.

Sindbis virus and Semliki Forest virus (SFV) have been extensively studied as the prototype of togaviruses, since these viruses are only weakly pathogenic to human.

**Classification:** Family Togaviridae is constituted by two genera: genus *Alphavirus* and genus *Rubivirus* (Table 13.2). Chikungunya virus, an emerging virus, is an important human pathogen belonging to the genus *Alphavirus*. Togaviruses are important veterinary pathogens, and are transmitted via mosquitoes. Some of them are zoonotic viruses, including Venezuelan equine encephalitis virus (VEEV). Rubella virus is the only member of togavirus family that causes significant disease in human—German measles.

**Epidemiology:** Togaviruses are largely transmitted via mosquitoes. Chikungunya virus, an emerging virus, caused a massive outbreak in some countries in Africa including Kenya and Madagascar in 2004–2006. VEEV, a zoonotic virus, could infect birds, horses, and human. VEEV infection causes rash, fever, and encephalitis. Rubella virus, that causes German measles in human, is unique among togaviruses in that it is not transmitted via mosquitoes.

**Virion Structure:** Togavirus virions are small (70–80 nm), enveloped, icosahedral capsid inside (Fig. 13.3). Togavirus virion structure stands out in that not only the capsid but also the envelope has an icosahedral symmetric structure. Indeed, 240 molecules of E1/E2 dimer form an icosahedral structure having $T = 4$ symmetry. Likewise, 240 molecules of capsid proteins form an icosahedral structure having $T = 4$ symmetry. Togavirus is unprecedented in that the envelope as well as the capsid has a symmetric structure (see Fig. 2.9).

**Genome Structure:** Togavirus genome is a positive-strand RNA of 11 kb (Fig. 13.4). In addition to the genomic RNA that encodes a polyprotein for nonstructural proteins, a subgenomic RNA is expressed that encodes a polyprotein for structural proteins. The 5' terminus of RNA is capped, whereas the 3' terminus is polyadenylated. Note that the
capping at the 5′ terminus is facilitated by the viral capping enzyme (ie, nsP1) (see Box 16.3). The polyproteins are processed into individual proteins either by viral protease (ie, nsP2 protease) or by host proteases such as signal peptidase and furin.

13.3 CORONAVIRUS

Coronaviruses (family Coronaviridae) are enveloped, spherical, and about 120 nm in diameter and possess a single-strand RNA genome of approximately 30 kb.

Mouse hepatitis virus (MHV) is a prototype of coronavirus. MHV outbreak in animal laboratory facilities represents a serious concern. In some cases, the facility has to be closed for many years until reuse. On the other hand, human coronavirus has been considered clinically unimportant, until SARS (severe acute respiratory syndrome) coronavirus

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4. **Subgenomic RNA** It refers to a viral RNA that is smaller than the full-length viral RNA.
was discovered in 2003, as a newly emerging virus. More recently, the MERS (Middle-East respiratory syndrome) outbreak in the Middle East has drawn attention to human coronaviruses again (see Fig. 21.11).

**Classification**: Family Coronaviridae is subdivided into three genera: alpha, beta, and gamma coronaviruses (Table 13.3). SARS-coronavirus (SARS-CoV) and MERS-coronavirus (MERS-CoV) are classified as Beta coronavirus.

**Epidemiology**: Human coronaviruses were known to cause only mild respiratory infections, until the SARS outbreak. The SARS-CoV outbreak that occurred in 2003 has drawn attention, since it was fatal, causing SARS (see below). Moreover, a novel human coronavirus, MERS-CoV, is responsible for a new emerging respiratory infection that occurred in 2012 in Middle East countries, including Saudi Arabia (see Fig. 21.11).

**Virion Structure**: Coronaviruses are enveloped and contain a large helical nucleocapsid inside (Fig. 13.5). The viral envelope is studded with spike glycoprotein trimer (S), hemagglutinin-esterase dimer (HE), membrane protein (M), and envelope protein (E). In particular, the protruding spike protein (S) characterizes the crown shaped virion, which it was named after. “Corona” (crown) refers to the characteristic appearance of virions under electron microscopy with a fringe of large, bulbous surface projections creating an image reminiscent of the solar corona.

**Genome Structure**: The genome of coronavirus represents a large single-strand RNA of 27–32 kb (Fig. 13.6). It is the largest RNA genome among animal RNA viruses. The ORF1a and ORF1ab are translated as polyproteins, which are subsequently processed to 16 nonstructural proteins. In addition to the large genomic RNA, coronaviruses have

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### TABLE 13.3 Classification of Coronavirus

| Genus         | Virus Species                     | Host       | Disease                                 |
|---------------|-----------------------------------|------------|-----------------------------------------|
| Alpha Coronavirus | Human coronavirus-229E               | Human      | Colds, pneumonia                        |
|               | Human coronavirus-NL63             | Human      | Colds, pneumonia                        |
|               | Transmissible gastroenteritis virus (TGEV) | Swine      | Gastroenteritis                         |
| Beta Coronavirus | Mouse hepatitis virus (MHV)         | Rodents    | Hepatitis                               |
|               | Human coronavirus-HKU1             | Human      | Pneumonia                               |
|               | SARS-coronavirus (SARS-CoV)        | Human      | Pneumonia, Gastroenteritis              |
|               | MERS-coronavirus (MERS-CoV)        | Human, Camel | Respiratory infection                   |
| Gamma Coronavirus | Avian infectious bronchitis virus (AIBV) | Avian      | Kidney infection                        |
eight subgenomic RNAs, each of them encoding one structural protein. In particular, the S (spike) envelope glycoprotein binds to the host cell receptor and determines tissue tropism and host range.

*SARS-CoV*: SARS outbreak first occurred in Southern China (including Hong Kong), and spread to South East Asia and Northern America within a few weeks (see Fig. 21.10). The initial casualty and media hype made the public paranoid, canceling overseas travel and international meetings. SARS outbreaks resulted in over 8000 infections and 700 deaths in 20 countries. It turned out that bats were the reservoir for SARS-CoV. It is believed that bat coronavirus had acquired the ability to infect human, extending the host range, by having a few mutations in the spike protein. Recently, *ACE* (angiotensin-converting enzyme 2) was identified as the cellular receptor for virus entry of SARS-CoV to human infection.
**MERS-CoV**: MERS outbreak first occurred in the Middle East (mainly Saudi Arabia) in 2012, and spread to European countries in a limited manner (see Fig. 21.11). As of June 2015, MERS-CoV caused 1266 cases and 470 deaths reported in multiple countries. MERS-CoV cases have been reported in 23 countries, including Saudi Arabia, Malaysia, Jordan, Qatar, Egypt, the United States, and South Korea. The fatality of MERS-CoV is considerably higher than that of SARS-CoV, approaching 30%. It is speculated that the virus spreads from bats to human via dromedary camel (see Fig. 21.11). The risk of sustained person-to-person transmission appears to be very low. Recently, dipeptidyl peptidase 4 (DPP4 or also known as CD26) was identified as the cellular receptor for virus entry of MERS-CoV. Further, a mouse model for MERS-CoV infection was established that expresses the DPP4. It is hoped that the established mouse model will facilitate the development of a vaccine and antiviral drugs.

### 13.4 PERSPECTIVES

In this chapter, three miscellaneous positive-strand DNA viruses are described. Noroviruses, a prototype of caliciviruses, are responsible for almost all viral gastroenteritis outbreaks worldwide. Nonetheless, no vaccine and antiviral drugs are available to control norovirus infection. The lack of susceptible cell lines and animal model for norovirus infection have imposed barriers to basic research until recently. A recent successful demonstration of human norovirus infection using B lymphocytes (see Journal Club) deserves more attention in this regard. Such progress in norovirus infection system will greatly advance our understanding on the infection pathology of human norovirus and at the same time facilitate antiviral drug discovery and preventive vaccine development. Sindbis virus and SFV have been extensively studied as the prototype of togaviruses. Surveillance on zoonotic togaviruses, such as VEEV, has become more important. Finally, the emergence of deadly human coronaviruses—SARS-CoV and MERS-CoV—have bolstered research in these viral and often zoonotic pathogens. Accordingly, great advances, such as identification of host cell receptor, the establishment of reverse genetics, and small animal model for infection, have been made in the past decade.

### 13.5 SUMMARY

- **Calicivirus**: Noroviruses, the prototype of caliciviruses, are responsible for almost all viral gastroenteritis outbreaks worldwide.
- **Togavirus**: Sindbis virus and Semliki Forest virus (SFV) have been extensively studied as the prototype of togaviruses. Chikungunya virus, an emerging virus that caused an outbreak in Africa during 2004—2006, belongs to the togavirus family.
- **Coronavirus**: Coronaviruses possess the larger RNA genome of approximately 30 kb. SARS-coronavirus was discovered as a newly emerging virus that caused the 2003 SARS outbreak and 2012 MERS outbreak.

### STUDY QUESTIONS

13.1 Consider that a novel positive-strand RNA virus was discovered. The RNA genome structure is organized similar to that of picornaviruses, encoding one large polyprotein. Moreover, a viral protein is covalently linked to the 5’ terminus of the RNA genome. (1) Please hypothesize the role of the 5’ terminus-linked viral protein. (2) How would you test your hypothesis?

13.2 Sindbis virus has subgenomic RNA as well as genomic RNA. (1) State your hypothesis on the mechanism by which the subgenomic RNA is transcribed. (2) How would you test your hypothesis?

### SUGGESTED READING

Jones, M.K., Watanabe, M., Zhu, S., Graves, C.L., Keyes, L.R., Grau, K.R., et al., 2014. Enteric bacteria promote human and mouse norovirus infection of B cells. Science. 346 (6210), 755–759.

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• Jones, M.K., Watanabe, M., Zhu, S., et al., 2014. Enteric bacteria promote human and mouse norovirus infection of B cells. Science 346 (6210), 755–759.

Highlight: The biggest hurdle in norovirus research has been that norovirus is not cultivatable in a cell culture. It has been speculated that noroviruses primarily target intestinal epithelial cells, which line the intestine and protect it from pathogens. Surprisingly, the authors here demonstrated that human norovirus can be propagated in B cells, with the help of enteric bacteria. It is an unprecedented finding that bacteria facilitates the virus infection.