Coronaviruses

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1 Introduction

Coronaviruses of humans have been classified as a subfamily of the Coronaviridae family. The viruses are roughly spherical, enveloped particles 120–160 nm in diameter. Their name derives from the characteristic "crown"-like projections on their surface, approximately 20 nm long. They are positive-sense, single-stranded RNA viruses, are sensitive to heat and lipid solvents, and have a distinct replication strategy common to other viruses in the order Nidovirales [1–3]. Coronaviruses have in the past been divided into three groups. In part, because of increasing work on coronavirus discovery in the wake of the outbreak of severe acute respiratory syndrome (SARS) in 2003, a number of new coronaviruses of humans (hCoVs) have been identified. This has resulted in the three groups being reclassified as genera of the Coronavirinae subfamily. Before the SARS outbreak, while it was recognized that the coronavirus infected many different species, particularly domestic and laboratory animals, their extreme diversity in nature was not fully appreciated. Although severe diseases of differing characteristics were known to occur in animals, in humans the viruses were thought to cause only acute respiratory infection which were generally mild. All this changed with the recognition that SARS was caused by a coronavirus (SARS-CoV). This signaled that coronaviruses could produce lethal disease and encouraged more broadly based research on the agent and its prevention and control. In the process, two new respiratory or endemic coronaviruses have been identified as causing human infections, which appear to resemble in epidemiologic characteristics those previously known. Very recently, MERS (Middle East respiratory syndrome) coronavirus, a novel human betacoronavirus lineage C virus, has been discovered zoonotically infecting humans in Middle Eastern countries.

In light of the differences between the endemic respiratory and the SARS coronaviruses in terms of epidemiology and clinical expression, they will be covered separately in much of this chapter.

2 Historical Background

Coronaviruses were first identified from domestic and laboratory animals before they were identified in humans. Infectious bronchitis virus of chickens was actually isolated in embryonated eggs in the 1940s. The late recognition of these viruses was, in large part, because of difficulty in recovering the human viruses using standard cell culture techniques [4]. The first human coronaviruses were identified by different techniques in the United Kingdom and the United States at approximately the same time. The British Medical Research Council's Common Cold Research Unit had been studying fluids collected from persons with natural respiratory infections by standard cell culture isolation methods and by inoculating them into human volunteers. Rhinoviruses or other cytopathogenic agents could be recovered from a portion of the fluids [5]. There was an additional, substantial portion from which no agents could be isolated but that could still cause colds in the volunteers. Organ cultures of human embryonic trachea or nasal epithelium were then used in an effort to detect the recalcitrant viruses present. A specimen, B814, that had been collected in 1960 from a boy with a common cold had not yielded a virus on inoculation into cell culture. After the specimen had been passaged serially three times in human tracheal organ culture, it could still cause colds on inoculation into volun-

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teers, which indicated that replication had taken place [6]. In Chicago during the winter of 1962, five agents were isolated in primary human kidney cell cultures from specimens collected from medical students with common colds. The viruses were ultimately adapted to WI-38 cultures and exhibited a type of cytopathic effect (CPE) not previously seen. A prototype strain, 229E, was selected for characterization and was found to be RNA containing, ether labile, and 89 nm in diameter but distinct serologically from any known ortho- or paramyxoviruses. Sera collected from the five medical students all exhibited a fourfold rise in neutralization antibody titer against 229E [7]. It became clear that these "novel" viruses were of more than passing significance when organ culture methods were added to standard cell culture techniques in a study of acute respiratory infections of adults conducted at the National Institutes of Health (NIH). Six viruses were found that grew in organ but not cell culture and were ether labile; on electron microscopy, the agents were shown to resemble avian infectious bronchitis virus (IBV) in structure, and these represented a distinct family of viruses [8]. The B814 and 229E strains were soon also demonstrated to have a similar structure on electron microscopy and to develop in infected cells by budding into cytoplasmic vesicles [9, 10]. As a result of the similarity of the human agents to IBV and also to mouse hepatitis virus (MHV), they were collectively considered to represent a group of vertebrate viruses distinct from the myxoviruses antigenically and structurally [11]. The name coronavirus was eventually adopted for the group to describe the fringe of projections seen around them on electron microscopy [1]. Except for 229E, none of the human coronaviruses had been successfully propagated in a system other than organ culture. McIntosh et al. reported successful adaptation of two of the NIH isolates, OC (organ culture) 38 and OC43, to the brains of suckling mice [12]. These strains were shown to be essentially identical antigenically but quite distinct from MHV. Only OC38 and OC43 could be so adapted; the other four OC strains resisted such attempts. The IBV was known to exhibit hemagglutination under certain conditions, but no such phenomenon had been demonstrated for the human strains until OC38 and OC43 were adapted to mice. Kaye and Dowdle found that the infected brain preparations would directly and specifically agglutinate red cells obtained from chickens, rats, and mice [13]. This technique greatly expanded the ability to do seroepidemiologic studies, since it was simple and reproducible.

Subsequent developments included adaption of OC38 and OC43 to growth in cell monolayers; either mouse brain or organ culture material could be used as sources of virus [9]. Not only was CPE available for reading of neutralization tests, but also the OC38 and OC43 viruses were found to hemadsorb red cells of rats and mice, making available a more precise means of evaluating endpoints in tests involving these organ culture-derived strains [14]. The other OC strains and B814 that could not be adapted to mouse brain resisted adaptation to cell culture as well; these distinct viruses have since been lost and may actually have been rediscovered recently.

Work on the coronaviruses of humans proceeded slowly, with debate about how frequently the viruses caused lower rather than upper respiratory disease. The methodological problems of working with them were a major impediment, as was their apparent involvement only in a relatively mild disease. All this changed when in 2002 SARS appeared first in China and then in other countries of the world. The near panic resulted because of its transmission characteristics, case fatality, and the fact that the agent was initially unknown. That last aspect was solved quickly with the identification of the causative agent as a new coronavirus. The knowledge that the virus emerged from a zoonotic reservoir spurred investigation of its possible source. Small mammals (civet cats) in live game-animal markets in Guangdong were identified carrying closely related viruses. This led to the identification of these animals as amplifier hosts and to the game-animal wet markets as an interface where zoonotic infection of humans was being initiated [15]. The natural reservoir was later identified to be Chinese horseshoe bats [16, 17]. The SARS epidemic ended following the use of various population control measures and unexpectedly has not recurred. However, increased attention to the coronaviruses continued globally. New coronaviruses were identified in various animal hosts, and two additional coronaviruses were identified in humans, the first since 229E and OC43. NL63 was identified in Amsterdam in 2004 from a 7-month-old child with febrile bronchiolitis [18]. The same virus was also isolated at almost the same time by the group in Rotterdam from an 8-month-old child with pneumonia [19]. The second virus, HKU1, was detected in a specimen collected from a 71-year-old man from China with pneumonia and then from another adult with the same diagnosis [20]. With the development of real-time PCR techniques for all four human respiratory coronaviruses, it has now become possible to identify them in many situations. Such identification is now typically done not only for these viruses but also for a variety of other respiratory agents. This has meant that coronaviruses are now detected as much in clinical settings as in epidemiologic studies.

3 Methodology

3.1 Sources of Mortality Data

Until the occurrence of SARS, coronaviruses of humans were not thought to cause death, except, possibly, in those with underlying conditions. This was in contrast to the situation in animals, where infections were sometimes fatal, depending on the particular virus. Since the SARS episode, the major change which has affected data on the respiratory coronaviruses has been more ready detection using the polymerase chain reaction (PCR) technique, so that infections are

now recognized in those who might previously have not been studied. This has especially been the case in hospitalized individuals, particularly those who will likely experience severe outcomes, such as the immunocompromised. It is also known from earlier studies that coronavirus frequently infects small children and reinfects adults, including persons with chronic respiratory disease [21]. It would be logical to assume that deaths could occur in these most susceptible segments of the population, but they are probably not very frequent.

A problem during SARS was in defining the specificity of infection, whether inapparent, mild, or fatal. This was related to the lack of a readily available diagnostic test in many areas where outbreaks occurred. To a large extent, cases were classified using a clinical case definition. In some cases that survived, there was an attempt at serological confirmation.

3.2 Sources of Morbidity Data

Since the coronaviruses of humans, other than the SARS virus, usually produce illnesses indistinguishable from those caused by other respiratory viruses, it is not possible to define morbidity in the absence of laboratory identification. Before PCR became available, it was difficult directly to identify the infecting virus; thus, besides anecdotal reports, most of the epidemiologic studies were based on identifying rises in antibody titer. In contrast, since development of the PCR technique, direct identification has become relatively simple. However, this seeming advance has often been accompanied by the use of the method to determine the incidence of infection in population groups and to define characteristics of associated disease or even seasonality. The major problem is the short duration of many of the available studies and the concentration on hospitalized individuals. As a result, while it is possible to say these viruses can sometimes cause hospitalization and to infer the particular clinical diagnoses they produce, it is difficult to estimate what proportion of overall illnesses are severe. A small number of studies have been population based and have produced the only data available for determining the overall impact of illness [22–24]. While the older population-based studies were limited to 229E, OC43, or both, they are useful since there are few recent investigations that give the same information. They were conducted in different settings but in some cases contemporaneously which allows direct comparison. In fact, almost no studies include all four recognized coronaviruses and cover more than a single year. This makes it difficult to discuss year-to-year variation in frequency of activity as well as seasonality, as was possible with the older data. Such studies are therefore of continuing interest, at least as background, in determining the long-term occurrence of the viruses. These original investigations were typically broadly based, with coronavirus infection forming part of overall evaluations of the role of viruses in general in respiratory illnesses. As indicated in the selected listing in Table 10.1, a variety of different open and closed populations were used for these studies. The 229E strain was originally isolated from medical students in Chicago as part of a long-term study of respiratory illnesses in young adults [7, 25]. Employee groups were the source of specimens in the NIH [26, 27] and in the studies at Charlottesville, Virginia [30]. Infection was also evaluated in children's homes [28] and boarding schools [5], among military recruits [34], and among children hospitalized for severe respiratory illnesses in various parts of the world [26]. Serological methods were used to detect occurrence in persons with acute exacerbations of asthma [33] or chronic obstructive respiratory disease [21]. Patterns of coronavirus infection were identified among the general population residing in the Tecumseh, Michigan, community as part of a longitudinal study of respiratory illness [31, 32]. Also included in Table 10.1 are more recent studies using PCR to identify infection. The previous studies, based on serology, often did not characterize infections identified clinically; in fact, challenge studies of volunteers were employed early, to determine characteristics of illnesses, because of problems associated with direct isolation [35, 36]. The more recent studies were able to characterize the illnesses clinically but have other limitations as indicated above.

Table 10.1 Selected longitudinal studies on the epidemiology of coronavirus infection in humans

Location	Population	Virus studied	Years
Chicago, IL [25]	Medical students	229E	1961–1967
Washington, DC [26, 27]	Hospitalized children	229E, OC43	1962–1967
Bethesda, MD [26, 27]	Adult employees	229E, OC43	1962–1967
Atlanta, GA [28, 29]	Institutionalized children	229E, OC43	1960–1967
Charlottesville, VA [30]	Working adults	229E, OC43	1962–1970
Tecumseh, MI [31, 32]	General community	229E, OC43	1965–1970
Denver, CO [33]	Hospitalized asthmatic children	229E, OC43	1967–1969
N. and S. Carolina [34]	Military	229E, OC43	1970–1972
Nashville, TN, and Rochester, NY	Hospitalized children <5 years of age	4 viruses	2001–2003
Melbourne, Australia [23]	Preschool children	NL63	2003–2004
Edinburgh, Scotland [22]	Medically attended illnesses—general population	4 viruses	2006–2009

3.3 Serological Surveys

Relatively simple serological technique was available for the first two coronaviruses identified (229E and OC43), and surveys of antibody prevalence were carried out in various parts of the world. Many surveys formed a part of studies directed mainly toward determination of incidence of infection. Information on the prevalence of antibody was available for populations in the United States [27, 30, 31], the United Kingdom [36], Brazil [37], and other parts of the world. A special situation was determining the meaning of the presence in man of antibody against coronaviruses of animals. The finding of mouse hepatitis antibodies in military recruits and in children and adults from the general population was surprising when first described in 1964 [38]. It is now recognized that this did not indicate past experience with MHV but rather with human coronavirus strains known to cross-react with it. Similarly, antibodies in human sera against the hemagglutinating encephalomyelitis virus of swine and the coronavirus of calf diarrhea also appear to represent cross-reactions with OC43 or related strains [29, 39]. In contrast, in a survey of antibodies to avian IBV, none could be found in a military population. Low-level antibodies were detected only in a portion of subjects who had close contact with poultry [40]. The virus is not known to cross-react with the human strains. More recently, following a gap in active work on the epidemiology of coronaviruses, ELISA methods have been developed for at least some of the viruses [41, 42]. These have not been as much used as the older techniques, given the availability of PCR, and may not be as specific to the particular strain.

3.4 Laboratory Methods

3.4.1 Viral Identification

Laboratory diagnosis can be achieved by identification of the virus in clinical specimens, using virus isolation or antigen or molecular detection techniques. Identification of infection can also be accomplished by detecting a host antibody response. Relevant specimens for virus detection are typically respiratory specimens, such as nasopharyngeal aspirates, washes or swabs, nose or throat swabs, and, when available, endotracheal aspirates or bronchoalveolar lavages. In the case of SARS, in which disseminated infection may occur, viral RNA could also readily be detected in the feces and in the serum or plasma.

Although PCR-based methods are becoming the "gold standard" in diagnosis, virus isolation is indispensable for characterizing virus, studying pathogenesis, determining susceptibility to antivirals, and developing novel antivirals and vaccines. The human coronavirus 229E was originally isolated in cell culture and later adapted to roller culture monolayers of human embryonic lung fibroblast cells such as WI-38 and MRC-5. A cytopathic effect of small, granular, round cells appears at the periphery of the cell monolayer [7]. Although these cells can be used for the cell-adapted prototype 229E virus strain, primary isolation of new 229E-like agents remains difficult. The human embryonic intestine (MA177) semicontinuous cell line has been used for the primary isolation of other 229E-like viruses [26]. Human coronaviruses OC38 and OC43, not related to 229E, were originally isolated in organ cultures of human trachea or lung [6, 8, 43, 44]. These two strains are similar and have been further adapted to replicate in suckling mouse brain and to primary monkey kidney and BS-C-1 cell cultures [9, 12, 14]. Another cell system, LI32, a heteroploid human lung line, has been reported to be suitable for primary isolation of 229E, a related virus (LP), and the B814, the first-described organ culture agent [45, 46]. This last finding has not been confirmed by other workers [9]. Similarly, MRC-C cells have been used for 229E-like viruses and human rhabdomyosarcoma cells for propagating 229E and OC43 [47, 48].

Human coronavirus NL63 was initially isolated in African green monkey kidney cells (LLC-MK2) [18]. Human colon carcinoma cells (CaCo-2) have recently been shown to be more susceptible to NL63 infection and show more prominent cytopathic effect [49]. HKU1 was initially discovered in 2005 by "broad-range" (primers designed to detect all known coronaviruses, rather than being specific for known coronavirus types) reverse transcriptase PCR (RT-PCR) [20], and new isolates remain difficult to grow reproducibly in the laboratory. However, it has been successfully cultured in human ciliated airway epithelial cells (HAE) [50].

SARS-CoV was initially isolated in 2003 on African green monkey kidney epithelial cells (VeroE6) and in fetal rhesus kidney cells [51, 52] during the SARS epidemic in 2003. VeroE6 cells, which are deficient in interferon induction, continue to be the cells of choice for culturing SARS-CoV at present. Following isolation of this novel agent, electron microscopy and molecular methods (random primer PCR and virus detection arrays) followed by partial genetic sequencing led to its identification as novel coronavirus.

Recently, MERS, a novel human coronavirus of the beta-coronavirus lineage C, has been detected in patients from Middle Eastern countries with severe pneumonia and renal dysfunction. Vero and VeroE6 cells are suitable for primary isolation of this virus from clinical specimens [53].

Continuous transformed cell lines do not mimic the physiological state of cells in tissues in vivo, and this may be the reason why many coronaviruses are difficult to culture in vitro. The use of primary cells from the relevant species, cells differentiated in vitro in air–liquid interface cultures and ex vivo organ cultures (as was used in the early days of virology), may be needed for the isolation of more fastidious viruses, since some animal viruses are more readily isolated

in culture although they are species-specific in their in vitro growth characteristics, especially on primary isolation [54–57]. Embryonated egg culture has been used as a host system for avian coronaviruses [58]. However, none of the plethora of bat coronaviruses detected by RT-PCR have been readily cultured in vitro to date, even in primary bat epithelial cell lines [59].

3.4.2 Antigen Detection

Immunofluorescence tests on cells from the respiratory tract (e.g., nasopharyngeal aspirates or swab) using commercially available reagents [60] or polyclonal [61] or monoclonal antibodies to 229E and HKU1 [62, 63] have been reported, but these are not widely used. Such antigen detection tests can also be used for the diagnosis of SARS-CoV infection [62]. Several ELISA systems have been developed to detect coronaviruses including coronavirus 229E [64] and the nucleocapsid (N) or spike (S) proteins of SARS-CoV in respiratory samples [65, 66].

3.4.3 Molecular Diagnostic Methods

Since the majority of human coronaviruses cannot be readily cultured in vitro, reverse transcriptase PCR (RT-PCR) and real-time quantitative RT-PCR have become the methods of choice for detecting and quantifying coronaviruses in clinical samples and for discovering novel viruses. RT-PCR methods were used for the detection of 229E and OC43 viruses in clinical specimens [67]. There are now a number of commercial assays that detect a range of respiratory pathogens (including coronaviruses) by the use of a combination of PCR amplification together with nucleic acid hybridization in Luminex bead assay formats. These methods provide the opportunity for the rapid detection of a panel of over 15 respiratory viruses including a number of coronaviruses in a clinical specimen. However, the sensitivity is generally less than that provided by individual RT-PCR methods [68, 69].

Apart from detection of known coronaviruses, RT-PCR is useful in virus discovery and further characterization. This includes a wide range of coronaviruses in bats detected solely by such broad-range RT-PCR methods because these viruses cannot at present be cultured [70]. For example, the first identification of HKU-1 as a cause of human disease was initially based on detection of viral RNA in clinical specimens by broad-range RT-PCR with primers designed to detect all viruses within the coronavirus family [20]. NL63 was discovered using the VIDISCA (virus-discovery-cDNA-amplified restriction fragment length polymorphism) method [18]. Amplified sequences from RT-PCR permit viral genome sequence analysis, which sheds light on virus structure, characteristics, biological properties, phylogeny, host and tissue tropism, epidemiology, cross-species transmission, and drug design [53, 71–75].

3.4.4 Serological Tests

The demonstration of rising antibody titers between acute and convalescent sera to a specific viral antigen provides evidence of recent infection, while the detection of antibody in seroepidemiologic surveys provides evidence of past infection. Methods that can be used for serodiagnosis have included the complement fixation test, neutralization test, indirect immunofluorescent (IF) assay, and enzyme-linked immunosorbent assay (ELISA). Hemagglutination-inhibition (HI) and gel-diffusion tests are less frequently used nowadays.

Neutralization test can also be used on coronaviruses that only grow in organ cultures [8]. Neutralizing antibodies could be detected as early as 5–10 days after symptom onset during SARS infection [76]. The seroprevalence for SARS-CoV in asymptomatic children and adults living in high- and low-risk regions in Hong Kong in 2003 showed that subclinical infection was rare [77, 78]. Pseudotyped virus expressing the SARS spike protein can also be used for detecting neutralizing antibody to SARS-CoV without the need for handling the live SARS-CoV, which has to be carried out in BSL-3 containment [79].

Indirect immunofluorescence tests use virus-infected cells fixed to inactivate virus infectivity as the solid-phase antigen to bind antibody in serum specimens. Anti-SARS-CoV antibodies present in the serum would bind to the viral antigens expressed on the infected VeroE6 cells; these primary antibodies could then be detected by adding secondary antihuman antibodies labeled with FITC [80]. IgM subclass antibodies to SARS-CoV, though declining in titer, can be detectable for more than 6 months after onset of disease. There is less of a decline in titers of IgG antibodies and neutralizing antibodies to the virus. Such assays can also be adapted to detect low- and high-avidity IgG antibodies both for discriminating early from late antibody responses and for distinguishing anamnestic cross-reactive antibody responses from primary specific responses. This may be useful in some clinical situations [81].

ELISA assays have been also developed for detecting antibodies to coronaviruses [82, 83] and have been used to investigate the epidemiology of coronavirus infections [84]. The elicited antibodies detected by ELISA predominantly react with the viral surface proteins rather than the ribonucleoprotein [85], and infections of 229E- and OC43-like viruses can be distinguished in ELISA assays [86]. ELISA has been used to study the seroprevalence of HKU1, showing an increase from 0 % in age <10 years old to a plateau of 21.6 % in the age group of 31–40 years old [87]. Recombinant protein-based ELISA assays for detecting antibody to SARS-CoV have been developed [88, 89]. The duration of antibodies by ELISA to the SARS-CoV spike protein was long-lived and paralleled neutralizing antibody responses, while those to the nucleoprotein was less long-lived [90].

A protein-based line immunoassay which individually detects antibody to HCoV 229E, NL63, OC43, HKU-1, and SARS-CoV nucleocapsid protein has been evaluated [91]. Paired sera from confirmed OC43 or 229E infections and 49 convalescent sera from SARS patients showed that there was considerable cross-reactivity between the two betacoronaviruses OC43 and HKU1 and between the two alphacoronaviruses 229E and NL63. However, 229E- or OC43-infected patients did not develop cross-reactive antibody to SARS-CoV. It is important to keep in mind such cross-reactions when evaluating the results of serological assays. It is also relevant that immunofluorescent assays appear to manifest the greatest cross-reactivity. Neutralization tests are likely to be the most specific although this has not been systematically studied with the recently discovered coronaviruses.

Historically CF or HI tests were used in epidemiologic studies of coronaviruses [7]. By this method, the CF test detected antibody in low titer and for only a short time after infection. However, if the antigen was highly concentrated, antibody could be detected at a higher titer, and this antibody persisted in the population so that the CF method could be employed in surveys of prevalence [92]. An indirect HI test for 229E virus using tanned sheep erythrocytes has been described which appears to be highly sensitive with no cross-reactions with OC43 virus [93]. CF tests can be satisfactorily performed with OC43 virus using infected suckling mouse brain as antigen [27]. The same mouse brain material can also be used in the HI test for OC43 antibody. In this test, the hemagglutination titer has been higher for rat than for chicken erythrocytes but sufficient with the chicken cells so that HI testing could generally be employed; this is of particular importance in view of the wider availability of chicken erythrocytes and the spontaneous agglutination that often complicates working with rat erythrocytes. Serum to be tested did not require treatment with receptor-destroying enzyme but rather standard heat inactivation at 56 °C. The agglutination took place equally at various temperatures including room temperature [94]. It has also been possible to demonstrate precipitin lines on gel-diffusion tests with coronavirus antigens concentrated 10- to 50-fold. Two or three precipitin lines were observed by Bradburne [92] in tests with hyperimmune animal or human serum, but others have identified only one such line [94].

The neutralization, CF, HI, gel-diffusion, and immunofluorescent techniques have been used in the antigenic analyses of the older strains of 229E and OC43 [36, 95]. Cross-reactive antibody responses among hCoVs have been reported. When sera from individuals experimentally infected with 229E- and OC43-like coronaviruses, including organ culture viruses, were tested, they are found to cross-react within each group but not across groups. Thus, it is possible that the ELISA test with 229E and OC43 antigens may be able to detect infection with most, if not all, human 229E and OC43 coronaviruses [86]. However, persons with antibody to 229E and OC43 (most of the adult population) did not have cross-reacting antibody to SARS-CoV even in immunofluorescent tests, allowing these serological tests to be reliably used for diagnosis and seroepidemiology of SARS. Patients who had OC43 infections without prior exposure to SARS-CoV had increases of antibodies specific for the infecting OC43 virus but not to SARS-CoV. However, antibody responses to SARS-CoV antibody were sometimes associated with an increase in preexisting IgG antibody titers for human coronaviruses OC43, 229E, and NL63 by immunofluorescent assays. This probably reflects anamnestic cross-reactive antibody responses to coronaviruses to which the patient has had prior exposure (i.e., similar to the concept of “original antigenic sin”) [96, 97]. The cross-reactivity is less when purifying nucleocapsid proteins are used in ELISA or Western blot assays.

4 Biological Characteristics

4.1 Classification

All human coronaviruses (hCoVs) are enveloped, positive-strand RNA viruses and belong to the subfamily *Coronavirinae* in the family *Coronaviridae*, order *Nidovirales*. The subfamily *Coronavirinae* is further divided into three genera, *Alpha-*, *Beta-*, and *Gammacoronaviruses*, corresponding to the previous informal classification groups I, II, and III, respectively; there is also a recently recognized *Deltacoronavirus* genus [98, 99] (Fig. 10.1). The genus *Betacoronavirus* consists of four separate lineages, designated from A to D, which correspond to the former subgroup 2A to D. As viruses sharing more than 90 % amino acid (aa) sequence identity in the conserved replicase pp1ab domain are treated as the same species, *OC43* and *human enteric coronavirus* (*HECV*) are thus now regarded as one species (*Betacoronavirus 1*).

At present, only members of alpha- and betacoronaviruses are known to infect humans. They differ from each other in nsp1 protein, which is distinct in size and sequence (gammacoronaviruses have no nsp1). In addition, alphacoronaviruses commonly share an accessory gene designated as ORF3. The type species for alpha- and betacoronaviruses are *Alphacoronavirus 1* (equivalent to *porcine transmissible gastroenteritis coronavirus*, *TGEV*, in older literature) and *murine coronavirus* (equivalent to *mouse hepatitis virus*, *MHV*), respectively. The viruses that are human pathogens are the alphacoronaviruses *229E* and *NL63*, the betacoronavirus lineage A viruses betacoronavirus 1 (which comprises *OC43* and human enteric coronavirus which are now regarded as variants of the same species) and HKU1, and the betacoronavirus

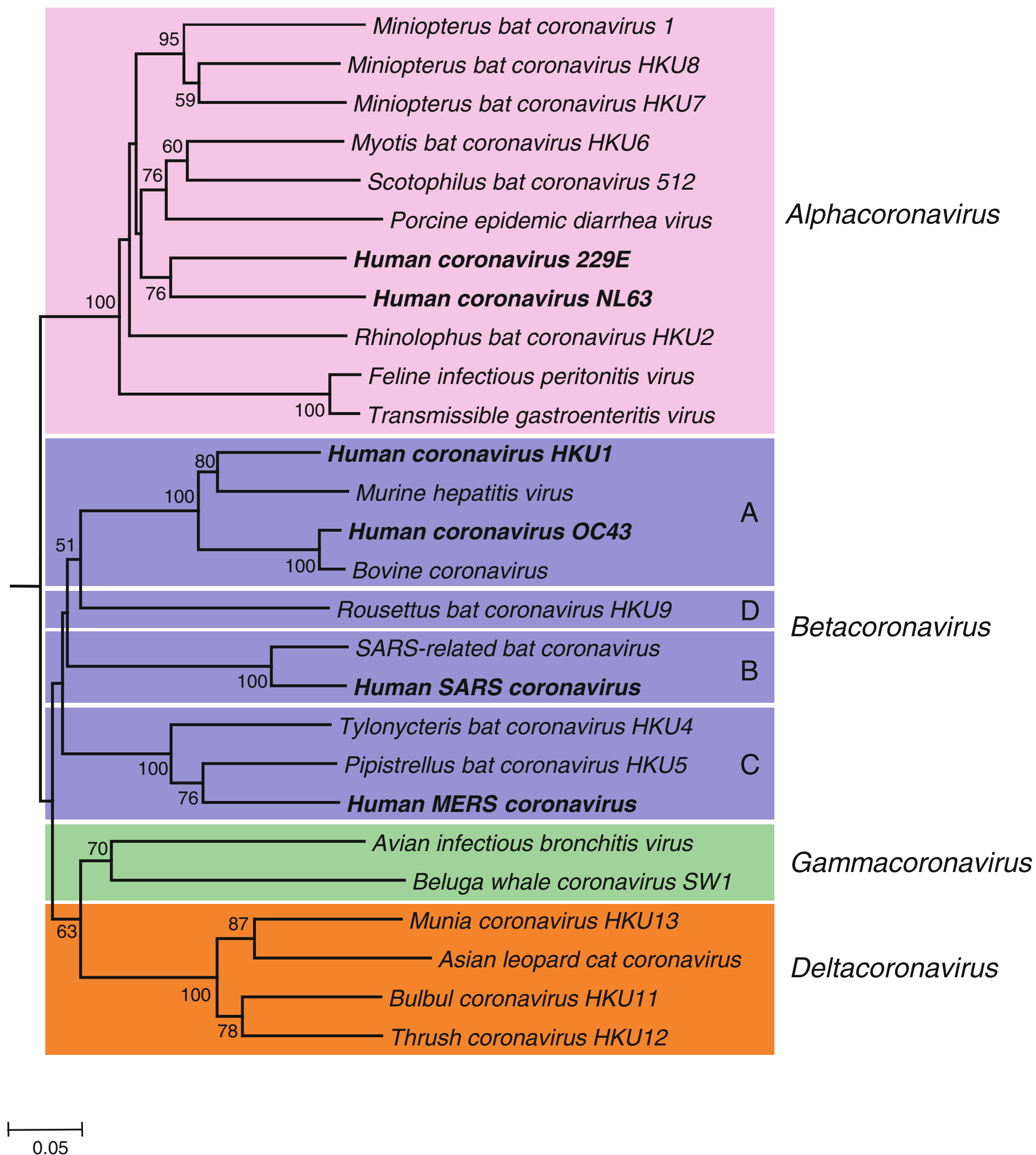

Fig. 10.1 Phylogenetic relationships of *Coronavirinae*. A rooted phylogenetic tree generated from nucleotide alignments of RdRp gene by neighbor-joining method with equine torovirus Berne as an out-group. Four genera of coronaviruses are indicated by different colors, *Alpha-* (*pink*), *Beta-* (*blue*), *Gamma-* (*green*), and *Deltacoronavirus* (*orange*). The distinct betacoronavirus lineages A through D were denoted. Human coronaviruses are denoted in *bold* (Figure is based on Refs. [1–3] and includes novel human betacoronavirus coronavirus 2c)

lineage B virus SARS-*related coronavirus* [98]. Recently, MERS (Middle East respiratory syndrome) coronavirus, a novel human coronavirus in lineage C, has been isolated. Phylogenetically, it is closely related to bat viruses previously detected in China and in Europe [53].

4.2 Genome and Structure

The name coronavirus comes from its appearance under the electron microscope with large 20 nm petal-shaped surface projections ("spikes") on a 120–160 nm spherical or

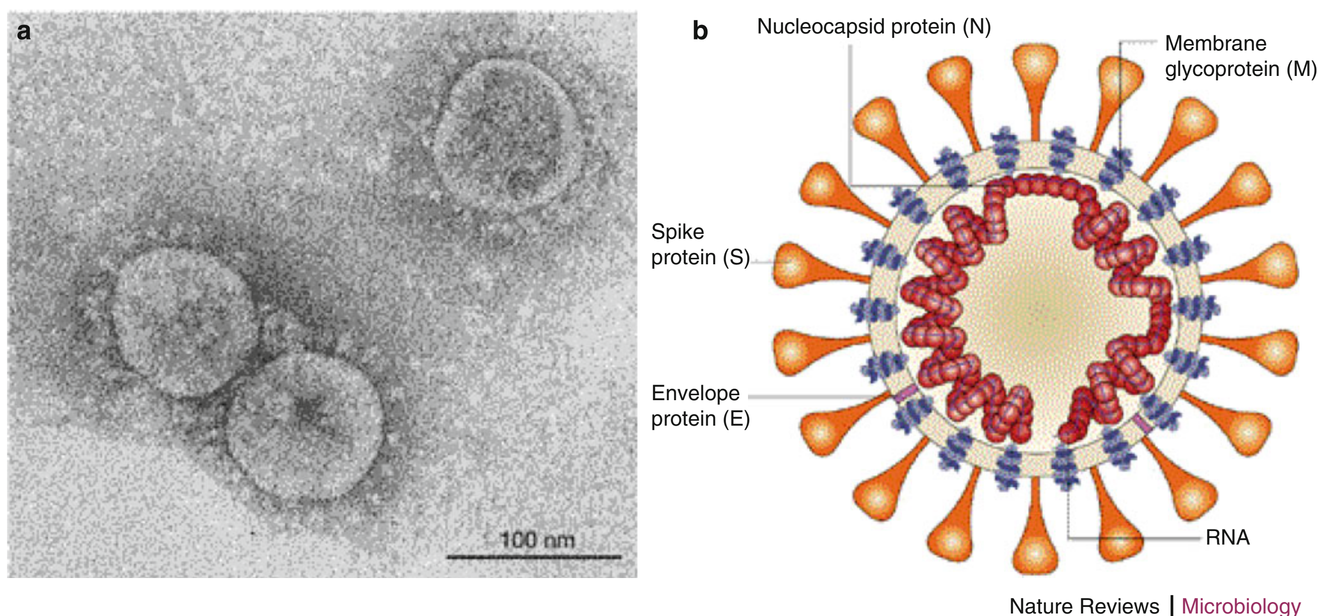

Fig. 10.2 Morphology of coronaviruses. (**a**) Electron micrograph of *severe acute respiratory syndrome-related coronavirus* (*SARS-related CoV*) cultivated in Vero cells. Large, club-shaped protrusions (*spike protein*) form a crown-like corona that gives the virus its name (Image courtesy of Dr L. Kolesnikova, Institute of Virology, Marburg, Germany). (**b**) Schematic representation of the virus. A lipid bilayer comprising the spike (*S*), membrane (*M*), and envelope (*E*) protein cloaks the helical nucleocapsid, which consists of the nucleocapsid (*N*) protein that is associated with the viral linear positive-stranded RNA. The lipid envelope is derived from intracellular membranes of the host cells [100]

pleomorphic body resembling a solar corona [100] (Fig. 10.2). Lineage A betacoronaviruses display an additional surface projection, the 5–7 nm homodimeric hemagglutinin-esterase (HE) glycoprotein. The interior ribonucleoprotein looks like either a long strand with 1–2 nm diameter or a helix condensed into coiled structures with 10–20 nm diameter. The virions are sensitive to heat, lipid solvents, nonionic detergents, formaldehyde, oxidizing agents, and UV irradiation [98].

The genome of coronaviruses consists of a linear positive single-stranded RNA between 26 and 32 kilobases and is the largest RNA virus genome to cause infection in humans [101]. The infectious genome has multiple open reading frames (ORFs), six of which are conserved across the subfamily and are arranged (in 5′–3′order) as ORF1a and 1b (which encode for two huge polyproteins, pp1a and pp1ab) and the ORFs for structural proteins, spike (S), membrane (M), envelope (E), and nucleocapsid (N). The two polyproteins pp1a and pp1ab from ORF1a/b are autoproteolytically cleaved into about 16 nonstructural proteins (nsp) which subsequently form into the replicase. Between the structural proteins S, M, E, and N lies the ORFs coding for the accessory proteins whose function is not essential for virus replication in vitro and the functions of many of them in vivo are still unknown.

As a positive-sense RNA genome, genome of coronaviruses serves as template for both replication and viral protein synthesis. Following entry into the cell by receptor-mediated endocytosis and uncoating of the virus genome, ORF1a/b of the genome is first translated to generate replicase proteins. These replicases use the positive viral genome as the template to generate full-length negative-sense RNAs, which in turn serve as templates for generating additional full-length genomes (i.e., replication). In viral protein synthesis, the 3′ proximal genes of the viral genome are first transcribed to segmented subgenomic negative-sense RNAs by discontinuous minus-strand RNA synthesis. The process is initiated at the 3′ end of the genome and proceeds until they encounter one of the transcriptional regulatory sequences (TRS) that reside upstream (5′) of most ORFs. Through base-pairing interactions, the nascent transcript is transferred to the complementary leader TRS, and transcription continues through the 5′ end of the genome. Therefore, all mRNAs of a coronavirus characteristically contain a common 5′ leader sequence fused to a downstream gene sequence. These subgenomic RNAs then serve as templates for positive-sense mRNA production and subsequent translation into viral proteins [101].

Three structural proteins S, E, and M are found on the viral lipid-membrane envelope; these are acquired as the virus buds into the endoplasmic reticulum, intermediate compartment, and/or Golgi complex of the host cell [98]. The spike protein (S) is a large homotrimeric type I membrane glycoprotein (1,128–1,472 aa). The S protein carries the receptor binding domain and is a class I fusion protein that triggers fusion between viral and host cell membranes

within the endocytic vesicle, thereby releasing the genome into the cytoplasm. The envelope protein (E) is a pentameric integral membrane protein (74–109 aa) that acts as ion channel for preferential transport of different ions into the virion. Although present in low copy number in a virion, E is significantly involved in virus budding, morphogenesis, and intracellular trafficking [102]. The membrane protein (M) is an integral type III triple-spanning membrane protein (218–263 aa). Being the most abundant protein in the viral envelope, it is essential in virus assembly within the infected cell. It could also interfere with host immune responses by inhibiting type I interferon production [103, 104]. The nucleocapsid protein (N) is a phosphoprotein that encapsidates the RNA viral genome to form ribonucleoprotein complex and regulates viral replication and translation (349–470 aa). It has RNA chaperone activity and also functions as an interferon antagonist [105]. In addition to these, the group A betacoronaviruses (includes OC43/HECV and HKU1) express an extra accessory homodimeric type I envelope glycoprotein, hemagglutinin esterase (HE), on its surface. It is related to subunit 1 of the influenza C virus hemagglutinin-esterase fusion protein (HEF) and mediates reversible virion attachment to *O*-acetylated sialic acids [106].

5 Descriptive Epidemiology

5.1 Incidence and Prevalence of Respiratory Coronaviruses

Coronaviruses are of major importance in common respiratory infections of all age groups. Before the identification of the newer endemic human coronaviruses, NL63 and HKU-1, it was recognized from the earlier studies that other unidentified human coronaviruses existed. It is still unknown if additional unidentified viruses exist, so that it is still useful to discuss burden in terms of both the individual virus and coronaviruses in general. Incidence of infection with 229E and OC43 exhibited a marked cyclical pattern, and reported rates can be expected to vary based on the number of seasons of high viral activity included in a particular study, again indicating the need for long-term evaluation. Table 10.2 presents a summary of results obtained in four studies which established the patterns of infection and illness [25, 28, 30, 31].

Another approach toward developing a minimal estimate of the total role of coronaviruses in respiratory illnesses comes from a study involving exhaustive laboratory examination, including organ culture, of specimens from 38 common colds. Coronaviruses were isolated from 18 % of the specimens, but an additional 13 %, which were negative in the laboratory, produced colds when given to volunteers [108]. Based on these results, which came from a limited age group, it has been estimated that coronaviruses could be responsible for at least 14 % of all respiratory illnesses in a general population [109].

Table 10.2 Reported frequency of infection or respiratory illness with 229E and OC43 in four locations

	Mean incidence of infection with	
Study	229E	OC43
Chicago medical students [25]	15/100/year	—
Tecumseh, MI [31, 32]	7.7/100/year	17.1/100/year
	Proportion of illnesses associated with	
	229E	OC43
Charlottesville, VA, employees [10]	1.7 % of illnesses	2.4 % of illnesses
Atlanta, GA, children [28, 108]	4.3 % of illnesses	3.3 % of illnesses

5.1.1 Incidence and Prevalence of 229E Virus

The frequency of 229E illness and infection was determined in several long-term investigations. The activity of 229E was found to be high in three out of 6 years of a study among Chicago medical students. The mean annual incidence of infection during the total period was 15 % where the criterion for identification was a reproducible twofold seroconversion determined by CF. There was marked year-to-year variation in infection frequency, ranging from a high of 35 % of those tested in 1966–1967 to a low of 1 % in 1964–1965. However, nearly 97 % of the infections occurred during the months from January to May, often at a time when isolation of rhinoviruses was at a low and seroconversions for 229E were only rarely accompanied by a rise in titer against another respiratory agent [25].

The serological study of 229E activity in the community of Tecumseh, Michigan, initially covered 2 years, which included one period of high prevalence. As with the study in Chicago, routine blood specimens were collected so that infection rates could be determined; however, the study group was composed of individuals of all ages living in their homes. Over the 2 years, infections were detected in 7.7 % of individuals tested by CF, as shown in the curve in Fig. 10.3. However, this appeared to be an underestimate of the actual activity of the virus. Serum specimens had been collected on a regular basis, 6 months apart; rises in titer by CF occurred most frequently in those pairs in which the second specimen was collected in April 1967, clearly indicating the peak period of viral dissemination. Both CF and the more sensitive N test results were combined to give an overall infection rate for the population studied; this rate, 34 %, was remarkably similar to the 35 % observed in Chicago at the same time. Because of the limited period of viral activity, it was possible to compare illness rates of those infected with persons not infected matched by age and sex; it was estimated that 45 % of the infections had

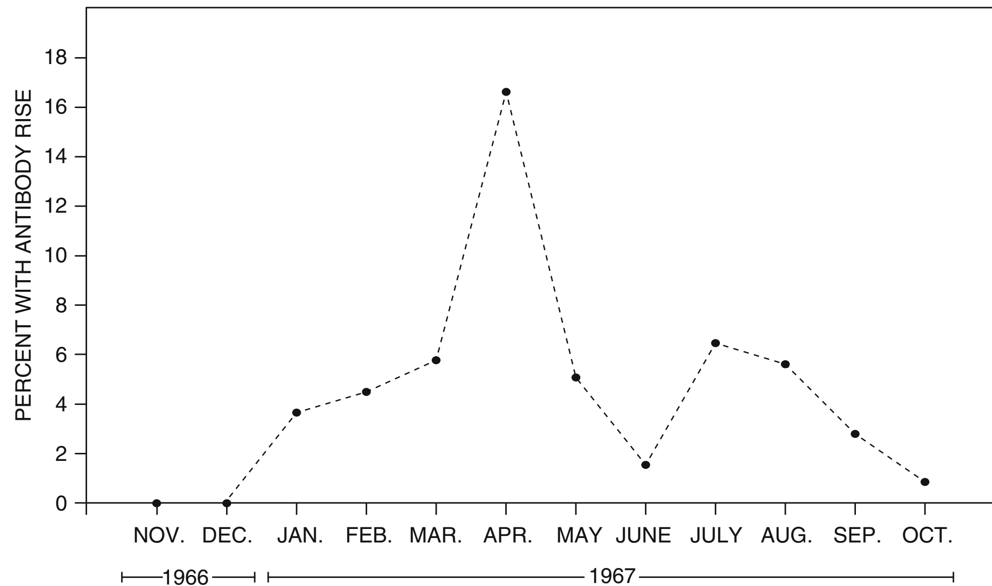

Fig. 10.3 Serological incidence by CF of infection with 229E virus in Tecumseh, Michigan, 1966–1967

produced clinical disease. Thus, the rate of 229E-associated illnesses during the outbreak was 15 per 100 persons studied. Activity in all age groups was apparent, including those under 25 years of age [31].

In other investigations of 229E activity, attention has been directed mainly toward study of associated illnesses; in such studies, sera have been collected before and after the illness rather than continually on a routine basis as done to determine infection rates. Employees at State Farm Insurance Company, in Charlottesville, Virginia, were studied during an 8-year period for rises in titer for both 229E and OC43. By CF, 229E infection could be related to 3 % of the colds that occurred in the winter–spring and to 0.4 % of colds that occurred in the summer–fall. There was some year-to-year variation in activity, but differences in the number of specimens tested from various years did not permit complete identification of cyclical patterns [30]. Employees of the NIH with respiratory illness were studied by both isolation and serology for 229E infection over a 6-year period. Again, attention was specifically directed toward certain segments of the 6 years, and no specimens were tested during other segments. Of particular interest once more is the segment from December 1966 to April 1967. Isolation of rhinoviruses and myxoviruses was uncommon at this time, but respiratory illness continued to occur. During this period, 24 % of those persons with colds studied had rises in titer for 229E. As part of the same investigation, paired blood specimens collected from infants and children admitted to the hospital with acute lower respiratory disease during the 1967 period of 229E activity were tested for rise in antibody against the virus, but none was found [26, 27]. Healthy children institutionalized in Atlanta, Georgia, were studied from 1960 to 1968; antibody response to 229E was determined by the indirect hemagglutinating test. The investigation involved collection of serum specimens related to illness and also routine collection of sera from some non-ill individuals. Frequency of infection showed marked variation from year to year. Overall, 4 % of colds could be associated with 229E infection, with greatest association in autumn, winter, and spring [28]. A more recent study took advantage of specimens sent from medical facilities in Edinburgh, Scotland, for laboratory identification of infection to study the incidence of a number of respiratory viruses identified by PCR. Overall, 229E was found in only 0.3 % of those sampled, lowest of any of the four coronaviruses studied. This may reflect that the source of the specimens was from illnesses seen in hospitals and primary care facilities, and coronaviruses are mainly involved in milder illnesses [22].

Surveys of prevalence of 229E antibody have been carried out to document past history of infection, often as parts of longitudinal studies. A general finding is that antibody is present in a significant portion of adults who, despite possessing this antibody, can subsequently experience reinfection and illness. Reports of antibody prevalence in adults in the United States have varied from 19 to 41 %, depending on the type of test used to determine antibody and the time of collection of serum [27, 30, 110]. Children under 10 years of age exhibited lower mean antibody titers than older children or adults [27, 31]. Individual sera from normal healthy adults collected serially in Britain from 1965 to 1970 were tested by Bradburne and Somerset. It is of interest that the proportion of sera positive by CF increased from approximately 17 % in specimens collected in October–December 1966 to 62 % in those collected in July–September 1967. This suggests that the spring 1967 outbreak that occurred in several parts of the United States may have taken place in Britain as well.

5.1.2 Incidence and Prevalence of OC43 Virus

Populations employed to study infection and illness caused by OC43 virus were generally been the same ones employed to study the occurrence of 229E virus. Kaye et al. [107] used the group of institutionalized children in Atlanta, Georgia, to identify infection by means of their HI test. Infections with the agent were detected in all years of the study, but with definite cyclical variation. Seasons most involved were the winter and spring. Overall, 3 % of the illnesses recorded in the 7-year period could be associated with OC43 infection, with a high of 7 % in 1960–1961. Interestingly, testing of the sera collected routinely from non-ill individuals indicated that an additional equal number of OC43 infections were occurring without the production of symptoms [107]. The Charlottesville study of adult employees was of both OC43 and 229E infections. Here, too, the emphasis was on illness, and in all years studied OC43 was associated with 5 % of colds in the winter–spring and with no illnesses in the summer–fall. Again, there was cyclical variation from year to year in the number of rises in titer detected [30].

The original isolations of OC38 and OC43 were made in December and January 1965–1966 as part of the study carried out among NIH employees with colds. Testing of sera collected from these employees indicated that during this period, up to 29 % of the colds studied were accompanied by rise in titer for OC43. In the children hospitalized with lower respiratory disease, up to 10 % of illnesses during this period were associated with such a titer rise. However, it was impossible to show that the relationship to disease was truly etiologic. This finding was in contrast to that seen with 229E, in which no rises in titer were detected in such cases [27, 111]. In the Tecumseh study, occurrence of OC43 infection was determined in the community population over a 4-year period: CF and HI tests were used on all specimens, and N tests were used as an aid in evaluating these results in selected specimens. During the total period, OC43-related infection was detected in 17.1 % of the 910 persons studied for 1 year. Most of the infections took place in the winter–spring months of 1965–1966, 1967–1968, and 1968–1969. The only winter–spring period without such activity was in 1966–1967, when the 229E outbreak had taken place. The 1968–1969 outbreak of OC43 infection was nearly as widespread as the prior 229E outbreak, with 25.6 % of the population studied showing evidence of infection. Of special note was the fact that children under 5 years of age had the highest infection rates [32, 112]. More recently, in the Edinburgh study involving medical care, OC43 was the most commonly identified coronavirus but only was identified by PCR in 0.85 % of specimens. This again may be a reflection of the source of the specimens. Surveys of antibody prevalence have been conducted in several settings using OC43 antigens. McIntosh et al. [27] found that children began to acquire antibody to this virus in the first year of life. By the third year of life, more than 50 % had antibody present. Among adults, 69 % could be demonstrated to have antibody; this indicates, in view of the high incidence of infection with the agents in all age groups, the frequency with which such infections must represent reinfection. The high prevalence of antibody has been confirmed in other studies [28, 30, 112]. In Britain, Bradburne and Somerset followed prevalence of antibody for OC43 over time, as they also had done with 229E [36]. Each year, the greatest prevalence of antibody was found in the winter–spring period. The single highest point in antibody prevalence was in January–March 1969, at the same time the OC43 outbreak was occurring in some parts of the United States [30, 38].

5.1.3 Geographic Distribution

Occurrence of coronavirus infection has now been documented, by isolation, PCR, or serology, throughout the world. In earlier studies, in the United States, in addition to the studies listed in the first part of Table 10.1, a 229E-like virus was isolated in California, and OC43 and 229E have been demonstrated to be present in many regions of the country [21, 113]. Extensive studies have been carried out by the Common Cold Research Unit, which have demonstrated the presence of the agents in Britain. The activity of 229E virus has been documented in Brazil in an early study of children and adults with and without respiratory illness. Significant rises in antibody titer accompanied respiratory infection in the nonhospitalized children. Prevalence of antibody was determined by CF, and like the situation in some studies in the north temperate zone, children had little antibody, whereas 26 % of adults were antibody positive [37]. Later investigations have confirmed the worldwide distribution of these agents [112, 114]. In particular, the widespread use of PCR has now allowed easy documentation of the activity of all the coronaviruses. In fact, one of the four viruses now recognized, HKU1, was first identified in the subtropical city of Hong Kong [20]. These findings suggest that coronaviruses are worldwide in distribution and cause similar types of illness in different localities [115], as has been noted with many other respiratory viruses [116, 117].

The newly recognized human MERS coronavirus infections have only occurred in Middle Eastern countries (Jordan, Qatar, Saudi Arabia, the United Arab Emirates) with limited secondary transmission being reported in France, Italy, Tunisia, and the United Kingdom. The infection is probably of zoonotic origin although other scenarios cannot be completely excluded at present. Persons with immunosuppressive conditions and other underlying diseases appear to be particularly susceptible to infection. While there have been significant clusters of infection in some health-care facilities, MERS coronavirus appears to have limited capacity for human-to-human spread at present [118–120].

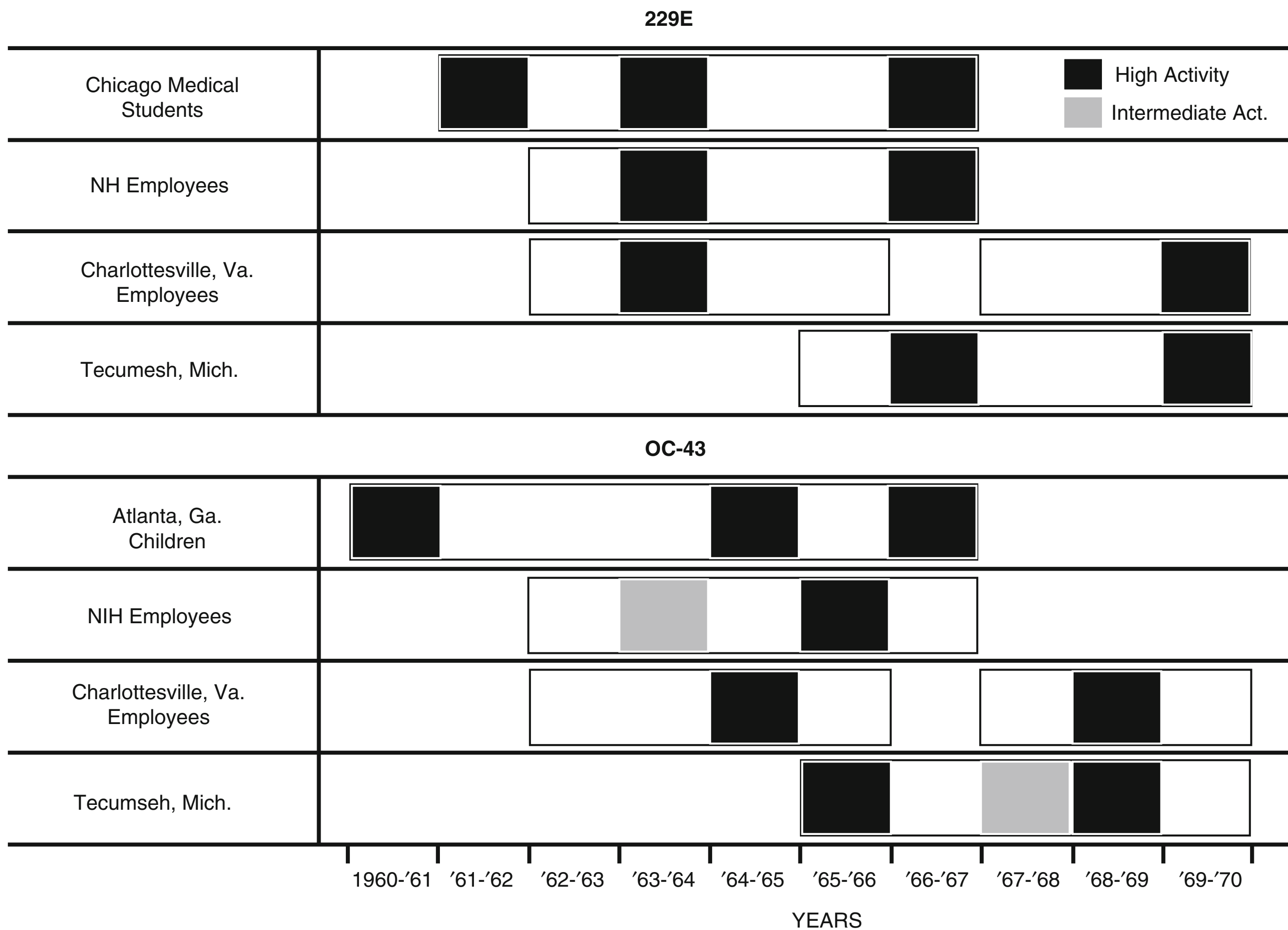

Fig. 10.4 Cyclic behavior of 229E and OC43 viruses observed in five longitudinal studies

5.1.4 Temporal Distribution

Because most illnesses caused by coronaviruses are similar to those caused by other respiratory viruses, it is impossible to identify epidemic behavior of the viruses clinically. In early epidemiologic studies, there was, however, evidence of variation in the frequency of infection on both a seasonal and a cyclical basis. In these investigations, isolation and rises in antibody titer for all types of coronaviruses were rare events outside the period from December through May. This is the portion of the year in which isolation rates for rhinoviruses often reach their lowest level. An exception to this rule was a study in which frequent rises in titer were detected by ELISA in summer as well [84]. More recent studies identifying the viruses by PCR have largely confirmed the winter seasonality of the viruses in the north temperate zone; one study observed that the timing of coronavirus identifications was similar to that of influenza in winter–early spring [22]. As expected, the seasonality appears to differ in places like Hong Kong, based on accumulating data [121].

In the earlier multiyear, population-based studies, a cyclical pattern could also be seen in the occurrence of individual virus types. In Fig. 10.4, data are summarized from five longitudinal studies of coronavirus activity carried out in different parts of the United States. In all studies, some sporadic activity did occur in nearly all years studied, but rises in antibody titers were concentrated in certain years in which they far exceeded the means for the entire studies. Those periods are indicated as solid black boxes in the figure. The times during which specimens were collected in each investigation are indicated in the figure by the white boxes. Activity of 229E was detected in all four studies at the same time, even though two were in the Midwest and two in the eastern United States. It seems possible, on the basis of these data, to postulate a 2- to 3-year cycle for this agent. The greatest number of infections in Chicago was seen in 1967, after the absence of the agent for 3 years; that pattern suggests a role of herd immunity in determining the time of reappearance of the agent.

With OC43, the situation is quite different. As with 229E, in no investigation did 2 years with high rates of infection or illness follow one another. A possible exception was in the Tecumseh study. However, the agent that caused the rises in titer in 1967–1968 did not appear as closely related

serologically to OC43 as the agent involved in the other two outbreaks. This observation indicates a problem in identifying cycling of OC43 using the serological test employed.

More recent studies using PCR have mainly not been population based or have focused on a limited number of the four known respiratory coronaviruses, so that comparable observations across all of the viruses are not possible. However, it is possible to conclude that, in any winter season, all four viruses may be identified in a single geographic area [122, 123]. There are likely to be increases of one or more in specific years, but it is unlikely that any coronavirus disappears completely; this is somewhat similar to our growing realization, with better surveillance, of the long-term occurrence of influenza types and subtypes [22].

5.1.5 Age

There is little available evidence that the respiratory coronaviruses behave differently than other respiratory viruses: infections are most common in children and decrease with increasing age. However, it is unclear whether the drop-off is modest or more extreme, as is the case with respiratory syncytial virus [124]. In the Tecumseh study, a total population group was followed. During the 1968–1969 OC43 outbreak, infection rates were relatively uniform for all age groups, varying from a high of 29.2 per 100 person-years in the 0–4 age group to 22.2 in those over 40 years of age [32]. The reversal of the pattern of age-specific infection rates customarily associated with the respiratory viruses becomes complete with 229E. Infection with this virus has been more difficult to demonstrate in small children than in adults. In Tecumseh, during the 1966–1967 outbreak, highest age-specific infection rates by CF were found among those 15–29 years of age, following a steady increase in infection frequency from the 0- to 4-year-olds. However, when neutralization tests were used to detect infection, the 15- to 19-year-olds still had high infection rates, but the serial increase to that point among younger age groups was much less steep [31]. This suggests that the apparent sparing of small children with 229E may be an artifact resulting from the relative insensitivity of the young to the serological procedures commonly employed. It would be surprising if two different coronavirus serotypes behaved so differently [125].

5.1.6 Other Factors

There is little evidence for or against a sex differential in infections with the coronaviruses. In Tecumseh, adult females experienced higher infection rates with OC43 than adult males, which is in conformity with the usual patterns of all respiratory illnesses [126]. Similarly, female volunteers appeared to be more susceptible to infection with 229E-like strains than males in artificial challenge studies [127]. In the study by Candeias et al. of antibody prevalence, the results were examined by sex, but no significant differences could be observed [43]. There are no data available on occupational or racial susceptibility to infection or on the role of socioeconomic status in influencing rates. Occurrence of infection in closed or special populations, such as military recruits or residents of children's institutions, has been reported [5, 26, 34]. The role of the school-age child in dissemination of coronavirus has not yet been clearly defined, but it would be surprising if these infections differed in their transmission pattern so markedly from that documented with the other agents. Because of the high frequency of infection in older children and adults, other sites of dissemination may also be of significance. It has been possible to show that the family unit is of importance in transmission, since clustering of 229E and OC43 infections in families was observed in the Tecumseh and Seattle studies [31, 128].

Although nutritional and genetic factors have not been associated with susceptibility to coronavirus infections, there are clear indications that the viruses are associated with exacerbations of chronic obstructive respiratory disease. Such a finding is hardly surprising in view of the high infection rates that have been observed in unselected older adults [129]. It has not yet been demonstrated whether this represents true increased susceptibility to infection or simply a more severe form of expression of the infection when it occurs in an already compromised host. In addition to the situation in older individuals, there is evidence that both OC43 and 229E may trigger acute attacks of wheezing in young asthmatics; in fact, in one study, coronaviruses were the most common agent involved in episodes of wheezy bronchitis [21, 33, 117, 130]. Recent studies using the PCR technique also associate the viruses with illnesses including pneumonia in immunocompromised patients [63, 115, 131]. One study identified all the viruses over the course of a year but the newer viruses, NL63 and HKU1, most commonly. Again, this may be a reflection of these viruses being most common at that point in time; it should be noted that shortly after the first identification of NL63 in one city in the Netherlands, it was again identified in another, which may indicate increased circulation at the time [18, 132].

5.2 Epidemiology of Severe Acute Respiratory Syndrome

In late 2002, the SARS coronavirus emerged in humans in southern China as a zoonotic pathogen [133]. Infection spread in Guangdong province for approximately 3 months before an infected individual visited Hong Kong in mid-February 2003. That case infected a number of tourists, sparking a global outbreak, and also went on to initiate a large outbreak in Hong Kong [73]. The subsequent global outbreak lasted around 4 months and had a substantial impact on global travel, trade, and economy [134]. Sustained epidemics have not occurred since 2003, although there have been a few sporadic events or minor outbreaks in Singapore,

Taiwan, and mainland China in 2003–2004, with most of them linked to laboratory releases and only four cases from mainland China perhaps of animal origin [135].

SARS patients initially developed influenza-like prodromal nonspecific symptoms including fever in the first week and usually presented cough, dyspnea, and diarrhea within 14 days. Severe illness developed rapidly progressing to respiratory distress and oxygen desaturation requiring intensive care and potentially resulting in death [136]. The World Health Organization (WHO) defined a suspected SARS case as a person with high fever (>38 °C) and cough/breathing difficulty, who either had close contact with a suspect or probable case of SARS or resided in or traveled to an area with recent local transmission of SARS in the 10 days prior to onset of symptoms. A suspected case became a probable case when (1) the patient's chest X-ray (CXR) presented infiltrates consistent with pneumonia or respiratory distress syndrome (RDS), (2) he/she was positive for SARS-CoV by laboratory assays, or (3) his/her autopsy findings were consistent with the pathology of RDS without an identifiable cause [137].

5.2.1 SARS Epidemiology in Time, Place, and Person

In total, 8,096 "probable" SARS cases were reported to the World Health Organization by August 2003 [138]. The most affected areas were Hong Kong, with 1,755 "probable" cases among a population of 6.8 million, and mainland China, with 5,327 "probable" cases among a population of 1.3 billion. Taiwan, Canada, and Singapore also experienced notable epidemics, with 346, 251, and 238 probable cases, respectively, while altogether cases were reported in more than 25 different countries and administrative regions. Reported cases of SARS globally and in Hong Kong by time of symptom onset are shown in Fig. 10.5.

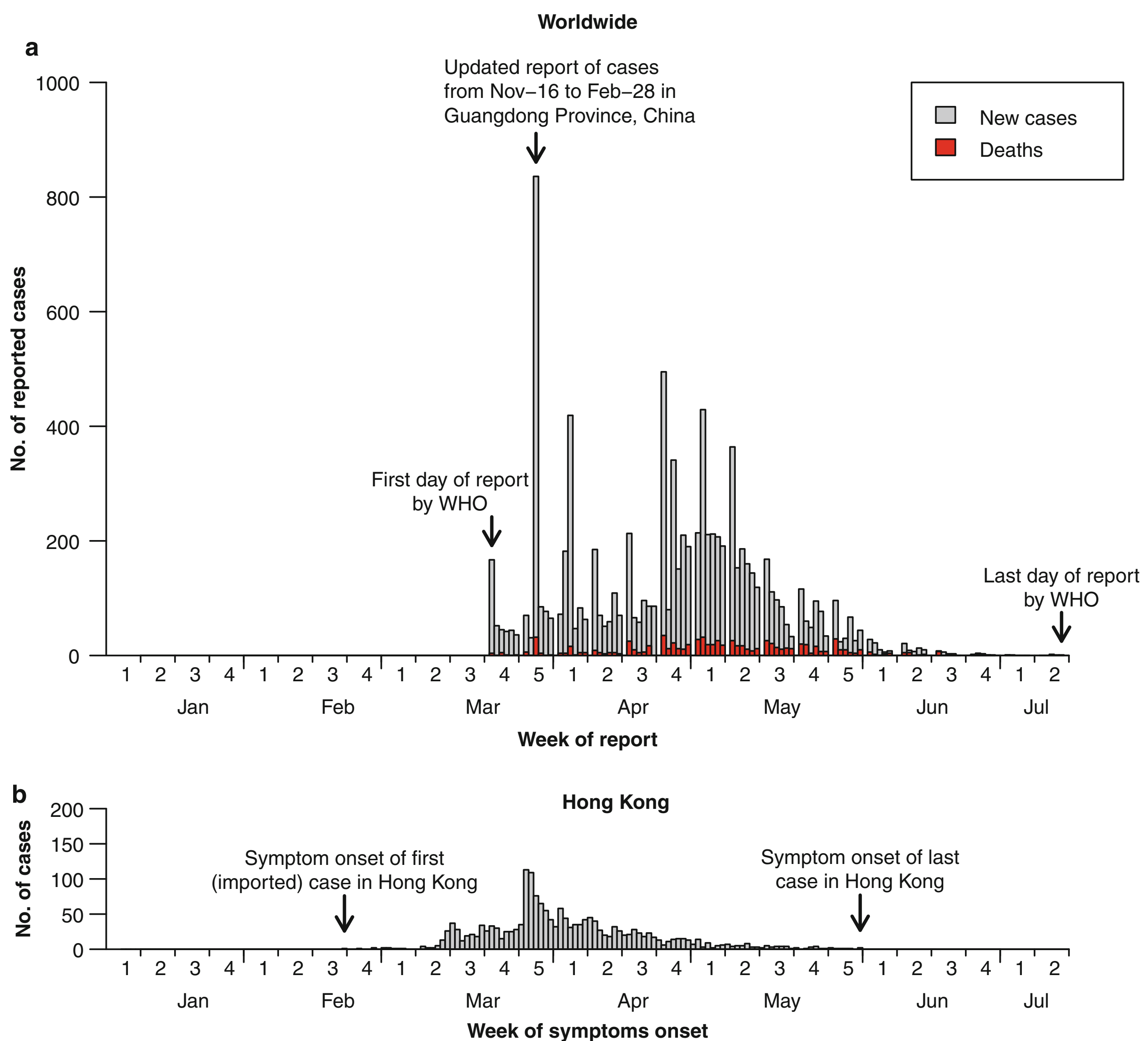

Fig. 10.5 Probable cases of SARS by week of onset (Source: [138]). (**a**) Cases worldwide, (**b**) Cases in Hong Kong

A common feature of SARS outbreaks in different regions was the central role of hospitals and transmission among patients and health-care workers [139–143]. A peak in infectiousness was thought to occur around 10 days after illness onset [144], by which time cases would have been hospitalized, and certain medical procedures were particularly prone to generating transmission [145]. Hospital transmission played a prominent role in the initial epidemic in Hong Kong, with more than 250 cases attributed to an outbreak at the Prince of Wales Hospital in early March 2003 [146, 147]. The Canadian outbreak began when a case returning from Hong Kong was admitted to hospital, and 72 % of cases were subsequently attributed to nosocomial transmission [143, 148].

Old age and the presence of comorbidities including diabetes mellitus, hypertension, coronary artery disease, and chronic obstructive pulmonary disease increased the risk of death or adverse outcomes, such as admission to an ICU requiring mechanical ventilation and development of ARDS [149, 150]. Sex (male), high lactate dehydrogenase concentration at presentation, and higher SARS-CoV viral load have been found contributing to higher case fatality rate as well [151]. Genetic factors may contribute to host susceptibility to SARS infection [78, 152, 153]. To date, there have been no studies on the relationship of race, socioeconomic status, occupation, or nutrition to susceptibility to SARS infection.

5.2.2 SARS Transmission Dynamics

Concerted efforts were made during the SARS epidemic to determine the transmission dynamics and thereby support control [154, 155]. Contact tracing exercises provided information to estimate the incubation period at around 5 days, with around 95 % of infections leading to illness onset within 10–14 days [156–158]. Early in the epidemic, delays between illness onset and admission to hospital were typically 5–7 days, and a measure of the success of public health control measures was the reduction in onset-admission intervals to just 1–2 days by the end of the epidemic [150, 157]. On average, patients remained in hospital for around 3–4 weeks [157].

The basic reproductive number, R_0, an estimate of the average number of secondary cases resulting from one infected case in a completely susceptible population, was estimated to be in the range 2–3 [154, 155, 159]. The average time between successive cases was around 8.4 days [155]. Due to these features taken together, in the early stages of outbreaks, the number of cases approximately doubled every week.

One issue of early controversy was the case fatality risk. Early in the epidemic, technical errors led to underestimation [160]. For example, on March 25, the World Health Organization reported the case fatality risk to be around 4 %, based on 49 deaths among more than 1,000 cases at that time [161]. This estimate was erroneously low because cases had not yet recovered, and some cases would subsequently succumb to the disease [160]. After the epidemic, the case fatality risk was estimated to be 9.6 % with 744 deaths among the 8,096 probable cases [135]. However, this masks substantial variability between affected regions, from around 7 % in Beijing to around 17 % in Hong Kong [150]. Reasons for variation remain unclear but could partly be attributed to case definitions, partly to case mix including age and underlying heath conditions [150] and partly to case management [162].

Few cases are thought to have been asymptomatic or subclinical. Serological studies were conducted in various groups including health-care workers, close contacts of cases, other patients, and the general community in affected regions, and a review of these studies found that the average seropositivity rate was just 0.1 % among more than 20,000 individuals [163].

5.2.3 Successful Control of the Global SARS Epidemic

Despite a basic reproductive number in the range 2–3, higher than influenza, the global epidemic of SARS was effectively controlled by appropriate nosocomial infection control measures. A range of interventions contributed to containment [164], including the use of engineering controls such as negative-pressure isolation rooms [143]; improved adherence to the use of personal protective equipment such as gowns, gloves, and masks [165]; as well as administrative measures including patient triaging and isolation, visitor restrictions, and establishment of dedicated SARS teams of staff [166]. The importance of strict infection control was illustrated particularly well by the experiences in Taiwan and Toronto, where control of the initial outbreaks was followed by complacency and subsequent second waves [167, 168]. Despite the importance of infection control strategies, there are also examples of individuals with SARS who were hospitalized where infection control practices were lax and yet their infection did not result in outbreaks [169]. Patient factors may also have had a role in the risk of transmission [170].

6 Mechanism and Route of Transmission

The four endemic respiratory coronaviruses are presumably transmitted by the respiratory route. It has been possible to induce infection experimentally in volunteers by inoculating virus into the nose [35, 44]. The virus is most stable at pH 6.0, and low temperature appears to protect it against varied relative humidity [171, 172]. No other route of transmission for coronaviruses seems involved in man, although animal coronaviruses are infectious by the fecal–oral route [57]. There is currently no direct evidence to aid in identifying the main mechanisms of transmission. However, it is possible to compare the epidemiologic behavior of the coronaviruses with that of other respiratory agents, the transmission mechanisms

of which have been more directly studied. Large-scale outbreaks of coronavirus infections have taken place, as in Tecumseh in 1967 [31]. This is much more analogous to the situation seen with influenza than to that with the rhinoviruses [173]. Rhinoviruses are thought to be transmitted by large droplet and may at times spread via fomites [174].

Unlike the situation with the SARS coronaviruses, there is no evidence that any animal reservoir or vector is involved in the maintenance of infection or transmission of the other respiratory human coronaviruses. There has been a report of antibody to avian IBV in the sera of poultry workers but not of controls, but no evidence of any further transmission [40].

The SARS coronavirus was thought to spread through a number of different modes, most commonly via direct close contact. Health-care workers involved in direct patient care duties often had the highest attack rates [175, 176], while contact precautions were effective in preventing transmission [165]. SARS coronavirus was capable of surviving on dried, inert surfaces and was found on some hospital surfaces [177], and indirect contact was implied in the infections of some nonmedical staff [178]. Although there is substantial evidence to support the role of transmission through droplet and direct contact and some evidence to support transmission by indirect contact, there is relatively little evidence for airborne transmission [179]. In one large community outbreak in Hong Kong, a computational fluid dynamics model was used to demonstrate that airborne transmission was consistent with the observed pattern of infections [180], although this hypothesis was not formally compared with other possible explanations. However, lacking other evidence of airborne transmission despite unprotected extended exposures in health-care settings [169, 181, 182], the World Health Organization classified SARS as a disease with "opportunistic airborne" transmission to indicate that the disease naturally spreads by non-airborne routes but under special environmental conditions may spread by the airborne route [183, 184]. Diseases that are spread by opportunistic airborne transmission do not require special airborne infection isolation measures, for example, negative-pressure isolation rooms, but special precautions are recommended for high-risk procedures.

7 Pathogenesis and Immunity

7.1 Etiology and Immunity

Data that demonstrate the etiologic role of coronaviruses in respiratory infections are derived from laboratory and field studies. Coronaviruses interfere with the action of cilia in tracheal organ culture, which suggests that they could have the same effect in vivo. Epidemiologic studies also have demonstrated association of 229E infection with disease. During the 1967 outbreak of 229E infection in Tecumseh, Michigan, illness was significantly more common among those with infection than among matched subjects without infection [31]. Similarly, 229E infection among Chicago medical students was statistically associated with illness when those with rises in titer were used as their own controls [25]. Furthermore, experimental inoculation of volunteers with strains of 229E and OC43 isolated in the laboratory has resulted in clinical illness, fulfilling Koch's postulates modified by Rivers [185] for attributing an etiologic role to a microbe as a cause of disease [35, 36, 47].

SARS-CoV has also fulfilled the Koch–Rivers postulates for association with the disease of SARS. The virus was detected in patients with SARS but not in those without disease, and the virus was detected at the site of the pathology, that is, lung [51]. The virus was isolated in pure culture from the lung biopsy of a patient with SARS, and experimental infection of cynomolgus macaques with this virus produced a comparable disease. A specific immune response to the virus was demonstrated, and the virus was successfully re-isolated from the site of pathology in the infected animal [186]. Fulfilling Koch–Rivers postulates for more recently discovered human coronaviruses has been more challenging because of the lack of suitable animal models that recapitulate the disease in humans [187], and their etiologic association with disease lies largely on epidemiologic grounds.

An important characteristic of the respiratory coronaviruses is their apparent high rate of reinfection, which in volunteers has now been documented to be possible within a year of prior infection [188]. In the Tecumseh study, 81.5 % of those infected with OC43 actually possessed prior N antibody [189]. Possession of circulating OC43 HI antibody among the Atlanta children did not appear to play a role in modifying severity of a subsequent illness [28]. With 229E virus, Hamre and Beem [25] demonstrated that the frequency of rise in titer detected by N was inversely proportional to preinfection levels of N antibody, which would indicate that this antibody exerted some protective effect. However, the importance of this N antibody could not be confirmed when infection was detected by CF. Thus, circulating N antibody as measured at present may bear a relationship to modification of infection, but this association is not a very strong one. Since coronavirus infections involve mainly the surface of the respiratory tract, it is likely that secretory IgA antibody plays a more direct role in protection; this had in fact been demonstrated with a swine coronavirus [190] and subsequently with 229E in humans experimentally infected [191].

7.2 Virus Tropism and Pathogenesis

Interaction between coronavirus spike proteins and host cell receptors determines specifically the host range, tissue

Table 10.3 Human coronaviruses and their major receptors

Human coronavirus	Major receptor	Receptor expression
229E	Aminopeptidase N (APN) [4]	Epithelial cells of kidney, intestine, and respiratory tract; granulocytes; fibroblasts; endothelial cells; cerebral pericytes at blood–brain barrier; synaptic junctions; macrophages; and dendritic cells [4, 193, 194]
Betacoronavirus 1 (OC43/ HECV)	9-*O*-Acetylated sialic acid-containing receptors [195]	Erythrocytes, neural gangliosides, gut mucins [193, 196]
SARS-related CoV	Angiotensin-converting enzyme 2 (ACE2) [197]	Lung alveolar epithelial cells, enterocytes of small intestine, arterial and venous endothelial cells, arterial smooth muscle cells [198]
NL63	ACE2 [199]	Same as above
HKU1	Unknown	Not known
MERS coronavirus	DPP4 (CD26)	Found in many tissues including the respiratory epithelium [200]

From the wide range of receptor expression, one could appreciate that coronaviruses could cause illness in many parts of the body, including the respiratory and gastroenteric systems [101, 201]

tropism, and pathogenesis [192]. Coronaviruses have a wide spectrum of susceptible host cell range, determined by the expression of the relevant receptors (Table 10.3). Related coronaviruses may use the same or similar receptors for entry, and major receptors for hCoVs include aminopeptidase (APN) and ACE2. Most alphacoronaviruses bind to APN with the exception of NL63 and SARS-CoV which binds to ACE2. Some of the betacoronaviruses (including OC43) attach to 4- or 9-O-acetylated sialic acids via the virus S and HE proteins. The HE protein also has enzymatic activity to cleave sialic acid linkages and thus serves to release virus from infected cells after replication is completed. Some human coronaviruses have other binding receptors that allow virus attachment to host cells, but these are not sufficient as functional receptors to mediate viral entry by themselves. These include calcium-dependent (C-type) lectins such as L-SIGN (**l**iver/lymph node-**s**pecific **i**ntercellular adhesion molecule-3-**g**rabbing **n**onintegrin), which may serve as a receptor for 229E and SARS [202, 203]. Recognition of a receptor from the same family in another host species is possible and may allow cross-species transmission. For example, 229E can use either human or feline APN but not porcine APN [201], and human and palm civet ACE2 serve as receptors for epidemic strains of SARS-CoV, but mouse and rat ACE2 do not [204, 205].

The receptor for MERS coronavirus has been identified to be DPP4 (also known as CD26), a protein that is widely conserved across mammalian species and found on the surface of several cell types, including the human upper airways. As with APN and ACE2, DPP4 is an ectopeptidase that cleaves amino acids from biologically active peptides [200].

The human coronavirus OC43 and bovine coronaviruses share close genetic similarity suggesting that they arose from a common ancestor less than 150 years ago [206]. Coronaviruses can undergo dramatic changes in tissue tropism and virulence within the same host. For example, porcine enteric transmissible gastroenteritis virus caused a severe enteric disease in pigs. A spontaneously occurring genetic mutation (deletion) occurring in the spike gene led to a change in virulence and was associated with a switch of virus tropism from the gastrointestinal tract to the respiratory tract [207].

7.3 Pathology and Pathogenesis

There is limited data on the pathology of coronaviruses other than SARS-CoV because these infections are generally mild. Electron micrographic changes from the nasal mucosa of a child with a coronavirus infection showed minimal pathological changes [208].

Although the clinically major pathology of SARS was that seen in the respiratory tract, SARS-CoV caused a disseminated infection with virus being found in the feces, urine, and plasma or serum [209]. Early disease was associated with desquamation of the alveolar epithelium and disseminated alveolar damage with hyaline membrane formation in the alveolar spaces. Viral antigen was demonstrated in alveolar and bronchial epithelial cells and alveolar macrophages [210]. In intestinal biopsy specimens of patients with SARS, virus infection of intestinal epithelium was demonstrated by electron microscopy with minimal cytopathic effect which is consistent with the watery diarrhea seen in these patients [211]. High serum levels of pro-inflammatory chemokines (CXCL10, IL-8) and cytokines (IL-1 and IL-6) suggested a role for immunopathology although it is uncertain whether these inflammatory responses are causally relevant or an epiphenomenon in the pathogenesis [212].

8 Patterns of Host Response

8.1 Disease Characteristics

The incubation period of coronavirus colds is relatively short. In studies involving volunteers, the mean period from inoculation of virus to development of symptoms was from

3.2 to 3.5 days, depending on the strain (range, 2–4 days) [35, 44]. Following exposure, the virus apparently multiplies superficially in the respiratory tract in a manner similar to that in which multiplication occurs in vitro. Nasal airway resistance and temperature of the nasal mucosa increase [213]. Virus excretion usually reaches a detectable level at the time symptoms begin and lasts for 1–4 days. The duration of the illness is from 6 to 7 days on the average but with some lasting up to 18 days. Serological response either to induced or to naturally acquired infection has been quite variable depending on the infecting strain and the serological test employed. For example, among those experimentally infected with OC38 or OC43 virus who had a cold produced, only 46 % had rises in titer by HI and 23 % by CF. Fewer than half of those infected with 229E showed a CF rise. It is not clear how the existence of titer or preinfection antibody affects the magnitude of the response detected by these tests. Rises in N antibody titer are easier to detect and have been found with sensitive techniques in all volunteers experimentally infected [36, 92]. The use of the ELISA test has given added sensitivity in antibody detection; it is not as yet clear if decreased specificity should be a concern.

The respiratory coronaviruses cause cold-like illness that on an individual basis is difficult to distinguish from illness caused by other respiratory viruses. They have also been reported to cause pneumonia and other severe respiratory infections, such as croup and bronchiolitis [115, 123, 214]. Most of the evidence of their involvement in severe disease comes from reports from hospitals of the identification of the viruses by PCR. It is thus impossible to say what proportion of infections, which appear to be common, that result in hospitalization are in fact caused by these viruses. Another problem recently encountered is the frequent identification by PCR of other viruses in those in whom a coronavirus is found [22]. This complicates determining the primary etiology. In induced infections in volunteers, the most prominent findings have been coryza and nasal discharge, with the discharge being more profuse than that customarily seen with rhinovirus colds [35]. Sore throat has been somewhat less common and in children has been associated with pharyngeal injection [215]. Experimental colds caused by B814 virus were about as severe as those caused by 229E; however, natural OC43 infections caused illnesses with considerably more cough and sore throat than did 229E infections [216]. The mean duration of coronavirus colds, at 6.5 days, was shorter than that seen in rhinovirus colds, at 9.5 days [35].

Clinical disease occurred in no more than 45 % of those infected with 229E in Tecumseh during the 1967 outbreak [31]. In Atlanta children, OC43 virus produced illness in about 50 % of those infected [28]. It is likely that with increase in age and concomitant experience with these agents, the ratio of clinically apparent to inapparent infection will decrease. As with other respiratory agents, a continuum of severity of symptoms exists among those in whom infection results in disease, and this may also be related to past experience with the viruses.

The mechanisms that lead to recovery from coronavirus infections have not been well defined. In volunteer studies, in persons infected with 229E-like strains, it is clear that symptomatic reinfection can occur after a period of about 1 year. It is not clear whether this is due to waning immunity or antigenic drift. Immunocompromised patients shed virus for a prolonged period and may sometimes be associated with a fatal outcome.

The novel MERS coronavirus initially presents with fever and myalgia, sometimes with gastrointestinal symptoms, rapidly progressing to severe viral pneumonia leading to respiratory failure, with renal dysfunction observed in some patients. Virus was detectable in the respiratory tract as well as the stool [119].

8.2 Viral Antigens Associated with Immunity

Virus-neutralizing antibodies are those that react with the virus S (and, where present, HE) protein although some antibodies against the virus M protein can also neutralize the virus in the presence of complement. Virus-neutralizing antibodies mainly bind to the N-terminal S1 part of the protein, which is also the part of the protein that manifests the greatest amino acid sequence variation. The removal of glycans from the S protein greatly reduces the binding of neutralizing antibodies. Antibodies to the S protein have also been associated with enhanced pathogenesis in feline infectious peritonitis virus, but such immunopathology has not so far been convincingly demonstrated with human coronaviruses. The nucleocapsid protein contributes to cell-mediated immune protection [209].

Neutralizing antibody responses to SARS-CoV appear in the second week of illness and peak at around 30 days of illness and antibodies remain detectable for many years. The major neutralizing epitope is in the region of the S protein amino acid residues 441–700 [98, 99, 209].

9 Control and Prevention

It is premature at present to think in terms of control of respiratory coronavirus infection by vaccination. Thus, preparation of vaccines using conventional types is impossible. The frequency of reinfection observed is so high that control by vaccination may not be practical, but it is possible that future studies may allow further characterization of truly protective antibodies. Work on vaccines for the animal viruses is in

progress, and these studies may help in understanding issues of protection. Chemoprophylaxis and related measures may be a more practical approach; it has been shown that recombinant α-interferon can prevent infections artificially produced in volunteers [217], and other approaches have been under investigation [25, 217–219]. There remains environmental control of infection; such efforts have rarely been useful for other respiratory agents, but they may be more efficacious if a practical barrier to transmission can be devised [220].

The situation is quite different in terms of the SARS-CoV, because of the severity of the disease produced. Here, the work on vaccines moved forward in the years immediately after 2003. Some of this activity of specific vaccine development continues but at a slower pace. Even when human cases were occurring, it was unclear how such a vaccine, if available, should be used, given the distribution of infection and occurrence of clinical disease. With disappearance of human cases, it has become even more difficult to decide on the appropriate vaccine target populations, except for those who might be exposed in a laboratory setting.

During the SARS episode, as no virus-specific antiviral agent was available, a variety of treatments were used, including the broad-acting antiviral agents ribavirin and interferon as well as corticosteroids. Because of the severity of the disease, many were used in combination, and it was difficult to say retrospectively whether any individually or in combination had a positive effect [162]. Corticosteroid therapy was associated with both short-term (secondary infections, increased viral load) and long-term (osteoporosis, avascular necrosis) adverse effects [221].

10 Unresolved Problems

The major problem in working with the respiratory coronaviruses has been solved with the development of RT-PCR. Previously, because of the difficulty in growing them in cell culture, epidemiologic and clinical studies had to rely on serology. While less limiting in epidemiologic studies, where regular blood collections can be scheduled, it sharply constrained the ability to identify the role of hCoVs in causing severe respiratory infections. Now we recognize the existence of four different coronaviruses, and there may be more that have not yet been identified. Paradoxically, the situation has now been reversed; there are many reports on the involvement of these agents in hospitalized cases, but epidemiologic studies involving all four hCoVs in different populations over time have been relatively scarce. It was previously thought that the viruses cycle in their appearance over a period of years, but recent evidence is lacking, especially pertaining to the newly identified viruses, NL63 and HKU1; however, there are still data suggesting that, in the temperate zones, the viruses are most active in late winter–spring. Ironically, with the PCR technique, it has become common to identify more than one virus from the same individual. This is not limited to the coronaviruses but includes many other respiratory viruses as well. There is a need to determine whether these are true coinfections or whether there may be asymptomatic or prolonged carriage involved. If they are real coinfections, there may be consequences of having more than one agent present during an illness.

The SARS-CoV emerged from a zoonotic reservoir and spread worldwide in a short period of time. We still do not know how and why this happened, and therefore we must be concerned that such an event could occur again. We do not have either vaccines or antivirals for the SARS-CoV; the need is probably greater for antivirals, given the severity of the illness and the question of how a vaccine would be used in the current situation. The recent identification of a novel coronavirus gives increased urgency to this need; it is likely that an anticoronavirus drug would be of use whatever the particular type involved. Overall, the epidemiologic lesson to be learned from SARS is the need for good surveillance at the animal–human interface. The virus was probably transmitting locally from human to human for months before it escaped to the rest of the world. If this had been recognized, there could have been earlier efforts to contain spread, which was effectively accomplished later, but only after much damage had been done.

The emergence of a novel MERS coronavirus with potential to cause severe human disease, though so far originating in the Middle East and manifesting limited human-to-human transmission including transmission within health-care facilities, is reminiscent of the emergence of SARS and is a concern for global public health [120].

The numbers of laboratory-confirmed patients with MERS continues to increase with 837 laboratory confirmed cases and 291 deaths being reported to WHO as of 23 July 2014. The median age of all cases is 52 years, but primary human cases (those who have no exposure to other confirmed cases) are older (median age 58 years) compared to secondary cases (median age 45 years). The majority of confirmed cases have underlying health conditions. All cases so far have a link to the Middle East with primary human infections reported from Jordan, Kuwait, Oman, Qatar, Saudi Arabia and the United Arab Emirates. Cases reported from outside the Middle East have either a history of travel to the Middle East or exposure to a patient who acquired infection from that region. Clusters of human cases and evidence of limited human-to-human transmission have been reported. To date, more than half of the secondary cases have been associated with health care settings, including health care workers. Health care workers appear to have less severe disease in general, although deaths have occasionally been reported [222].

MERS coronavirus (MERS-CoV) has been detected in nasal swabs of apparently healthy dromedary camels, and adult animals from the Middle East and Africa have high rates of sero-positivity [223, 224]. In some cases, infection of dromedary camel herds has preceded disease in humans in close contact with such animals [225]. However, in a large number of human cases, there appears to be no record of a history of contact with camels. The dromedary MERS-CoV virus appears genetically identical to those infecting humans, but abattoir workers with repeated exposure to potentially infected animals have little serological evidence of infection [223]. The geographic distribution of MERS-CoV infection in camels (including North East Africa) is wider than that reported in primary human cases reported so far. It remains to be seen whether this represents under-recognition of human cases in a wider geographic area. MERS-CoV antibodies have been detected in archived dromedary sera collected over two decades ago [226], and this may indicate that MERS is a recently recognised, rather than a newly emerging, disease. However, the possibility of a recent virus mutation that increased the risk of transmission to humans cannot be ruled out.

No specific validated therapeutic options or vaccines are available so far.

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