Review

Human coronaviruses: what do they cause?

Lia van der Hoek

Laboratory of Experimental Virology, Department of Medical Microbiology, Center for Infection and Immunity Amsterdam (CINIMA), Academic Medical Center of the University of Amsterdam, the Netherlands

Tel: +31 20 5667510; Fax: +31 20 6916531; E-mail: c.m.vanderhoek@amc.uva.nl

SARS-CoV, human coronavirus NL63 (HCoV-NL63) and HCoV-HKU1 were first described in 2003, 2004 and 2005 respectively. Nevertheless, discovery of three new human coronaviruses does not necessary represent a sudden increase in emerging infections by new coronaviruses. Only SARS-CoV has recently been introduced to the human population; the other two have been circulating in humans for a long time. HCoV-HKU1 and HCoV-NL63 are respiratory coronaviruses, are frequently found during lower and upper respiratory tract infections, have spread worldwide, and prefer the winter season. These characteristics do not differ greatly from the symptoms described for the 'old' viruses HCoV-229E and HCoV-OC43. This report presents an overview of the current knowledge of the four human coronavirus that are now circulating in the human population.

Introduction

Coronaviruses cause a variety of diseases in animals including respiratory tract and central nervous system disease and gastroenteritis, but in humans the coronaviruses are only proven to be associated with respiratory tract illnesses. The most aggressive human coronavirus is SARS-CoV, which causes severe acute respiratory syndrome (SARS), a lung disease often fatal in humans [1–3]. SARS-CoV probably originated from a wild animal reservoir, most likely bats [4,5], and was initially transmitted to humans via infected civets. Causing about 8,000 illnesses and at least 800 deaths in 2003, SARS-CoV clearly demonstrated the potential for a novel coronavirus to jump the species barrier to humans and cause high morbidity and mortality. Fortunately the epidemic was controlled by a highly effective global response that used the traditional public health measures of case isolation, contact tracing and selective quarantine. As a result, SARS-CoV is no longer circulating in humans. Nevertheless, there are at least four other HCoVs that are circulating globally in the human population, especially in young children. Two, HCoV-OC43 and HCoV-229E, were identified in the mid 1960s [6,7], whereas two others, HCoV-NL63 and HCoV-HKU1, were discovered recently [8,9]. HCoV-229E and HCoV-OC43 were tested for pathogenicity in human volunteers, which helped to demonstrate that these viruses cause common colds [10]. For the new viruses HCoV-NL63 and HCoV-HKU1 both a human test system and an animal model are lacking at present. In this review we will give an overview of the diseases that are reported to be due to infection by HCoV-229E, HCoV-OC43, HCoV-NL63 and HCoV-HKU1, the four human coronaviruses that are currently circulating.

HCoV-229E and HCoV-OC43: the handkerchief studies

The first cultured human coronavirus (B814) was obtained from a boy with a typical common cold [11]. In 1965 Tyrrell and Bynoe reported that the virus isolated from this boy could be cultured in organ cultures prepared from tracheal cells of human embryos [6]. Infection of these cells caused a mild cytopathic effect and a reduced ciliary activity [6]. The real proof of viral replication in the cultures and the fulfillment of Koch's postulates was obtained by inoculating the cultured virus into healthy adult volunteers. These volunteers developed a typical common cold. Fever was rarely observed, but there was often considerable malaise, and it was noted that "the nose often streamed with watery secretion" [6]. The numbers of used handkerchiefs per day were measured as an indicator of the severity of nasal secretion, and it was mentioned that with coronaviral infection the volunteers might use up to 120 paper handkerchiefs in 1 day. Other observations included little cough and no sputum and on average the disease cleared up in less than a week. Soon thereafter more coronaviruses were isolated from humans, and among

them was the HCoV-229E strain [7]. For HCoV-229E also the symptoms after inoculation of the virus into healthy volunteers were studied. There was no difference observed between the symptoms induced by B814 infection and those induced by 229E infection. Infection by either virus induced roughly the same symptoms, with similarly high numbers of handkerchiefs used, malaise and limited coughs. The high number of handkerchiefs was very typical for coronavirus infection, and was rarely observed during rhinovirus infection [10]. In 1967 McIntosh *et al*. described more coronaviruses isolated from National Institutes of Health employees with acute upper respiratory tract illness [12]. One of these isolates was HCoV-OC43. Inoculation of HCoV-OC43 (and all other OC strains: OC44, OC48, OC16, OC37 and OC38) into healthy adult volunteers resolved also that these viruses were a causative agent for common cold [13]. The observed common cold symptoms were similar to those described for B814 and HCoV-229E. Regrettably, quantitative analysis of the used handkerchiefs was not included in this study.

McIntosh *et al*. demonstrated that antibodies from an HCoV-OC43-infected person did not neutralize HCoV-229E, providing the first evidence that these viruses were serologically unrelated [14]. Based on serology and genetics the coronaviruses can be sub-divided into three groups. The group III viruses are found exclusively in birds, whereas viruses from group I and II have mammals as their hosts. HCoV-229E was regarded the prototype of the group I human coronaviruses and HCoV-OC43 the prototype of the group II human coronaviruses. The first diagnostic assays were based on serological surveillance studies. Infection by a coronavirus was diagnosed in the case of a significant rise in antibody titre. A survey of the symptoms observed in adults who displayed serological evidence of group I infection demonstrated that the colds were relatively mild when compared with the symptoms observed in a group II infection [15]. During group I coronaviral infections mainly nasal symptoms were observed, whereas colds caused by group II coronaviruses presented with nasal complaints, sore throat and cough.

Because the HCoV-229E and HCoV-OC43 strains were the easiest viruses to culture, follow-up studies between the 1960s and 1990s included only these strains. The other early isolates (OC16, OC37, OC38, OC44 and OC48) were unfortunately lost for follow up. Moreover, the first strain that was identified, B814, was regrettably lost for further investigations. Over the years HCoV-229E and HCoV-OC43 became the species names.

HCoV-229E and HCoV-OC43 infection in children and the elderly

In all studies of adult volunteers that were inoculated with human coronaviruses, no lower respiratory tract infections (LRTI) were observed. However, Bradburne *et al*. suggested in one of the first publications on HCoVs that the lower respiratory tract is more susceptible in young children [10]. Where healthy adults have only upper respiratory tract illnesses (URTI), children might endure LRTIs. The first study in children measured rises in antibody response during an LRTI. A rise in titre of antibody to the HCoVs was observed in 8.2% of cases, but also in 1/13 control children without respiratory symptoms (7.7%). The disease observed in the children with LRTI was predominantly bronchitis, but also laryngotracheitis (croup), bronchiolitis and pneumonia [16]. However, it is not certain whether the LRTI was caused by the coronaviral infection because a rising antibody titre can also be observed in healthy children without symptoms. In elderly people with acute respiratory tract illness, a rise in antibody titre is frequently observed (26%) [17]. Of these elderly people, 98% had upper respiratory symptoms and 43% also developed lower respiratory symptoms. However this latter study lacked a control group, so no firm conclusions can be drawn as to whether the coronaviral infections are the causative agent for the disease in these elderly people. This addresses an important issue: to connect a disease to a certain pathogen, control groups are needed. For healthy adults it is proven that the coronaviruses cause common colds. For the more vulnerable population – children, the elderly and immunocompromised persons – the type of symptoms caused by coronaviral infection is unknown. It is most likely that the symptoms will be respiratory disease, probably of a severe nature due to a hampered immune response in these groups. However, control groups must be included to rule out the possibility that coronaviruses are innocent bystanders, uninvolved in the pathogenesis of the disease. Van Elden *et al*. performed an elegant study which solved this matter for children [18]. They described the frequent detection of HCoV-229E and HCoV-OC43 in children with acute respiratory tract illnesses (11%), using a real-time reverse transcriptase-PCR format. In this study 273 control samples were included, mainly derived from asymptomatic bone marrow transplant recipients who did not suffer from respiratory illness. In the control group only one positive respiratory sample was detected (0.37%, $P<0.01$ [*t*-test]), thus providing evidence that HCoV-229E and/or HCoV-OC43 infection in children is associated with upper and lower respiratory tract illnesses that are more severe than the common colds described in healthy adults.

HCoV-229E and HCoV-OC43 and other diseases

Some studies have suggested that human coronaviruses, in particular HCoV-OC43, might be involved in gastrointestinal disease. Coronaviruses can be detected in stool samples, and antibodies directed to HCoV-OC43 are observed more frequently in children with gastroenteritis [19]. Yet none of the HCoV-OC43-inoculated volunteers

developed a gastrointestinal disease [10,13]. An explanation might be that immunocompromised adults or children can shed coronaviruses via the gastrointestinal route, even without a gastrointestinal disease.

Another theory is that coronavirus infections might play a part in multiple sclerosis. Mouse hepatitis virus, a murine coronavirus, is a close relative of HCoV-OC43 and causes a multiple sclerosis-like demyelinating disease in the central nervous system of rodents [20]. In 1980 Burks *et al*. cultured two coronaviruses from brain material obtained at autopsy from two multiple sclerosis patients [21]. Furthermore, multiple sclerosis patients showed slightly higher concentrations of serum antibody directed to coronaviruses than controls. Another report on multiple sclerosis patients presented 12 coronavirus-positive brain tissues among 21 multiple sclerosis patients, but also two positives in 21 control tissue samples [22]. With PCR-based methods the detection of HCoV-OC43 and HCoV-229E in brain tissue became more sensitive and it was demonstrated that RNA from these viruses can be detected in both multiple sclerosis patients and in controls, but at higher levels in the multiple sclerosis patients [23,24]. However, the higher frequency of coronavirus detection in some studies might not represent a causal relationship. It could correspond to an increased susceptibility of these patients to coronavirus infection in the brain, due to the damage to the blood–brain barrier.

Identification of HCoV-NL63 and HCoV-HKU1

In January 2003 a 7-month-old child was brought to an Amsterdam hospital with bronchiolitis. Diagnostic tests for all known respiratory viruses were negative, but a cytopathic effect on LLC-MK2 cells was apparent. Using the virus discovery cDNA-amplified fragment length polymorphism (AFLP) (VIDISCA) method we discovered a novel virus and the complete genome of the virus (named HCoV-NL63) showed that it was a novel group I human coronavirus [8].

A second research group in the Netherlands reported detection of essentially the same virus shortly thereafter. Fouchier *et al*. described a virus (which they named HCoV-NL) in a Vero-E6 cell culture supernatant [25] that was originally obtained in 1988 from an 8-month-old boy suffering from pneumonia. The similarity to the previously described HCoV-NL63 strain was very high (98.8% at the nucleotide level) and it can be concluded that these two virus isolates represent the same species.

Almost 1 year later, a third group described the identification of the same human coronavirus [26]. Using universal coronavirus primers, patient samples were identified with coronaviruses that did not match at the nucleotide level with HCoV-229E, HCoV-OC43 or SARS-CoV. These authors gave their virus the name 'New Haven coronavirus' (HCoV-NH), although the partial sequences of their isolates clearly show that the novel coronaviruses identified in New Haven, USA, are very similar to the isolates from the Netherlands (94–100% identical at nucleotide level), and thus represented the same species [27,28].

In contrast to HCoV-NL63, HCoV-HKU1 was discovered only once. In 2004 a 71-year-old Chinese man with chronic obstructive airway disease was admitted to a Hong Kong hospital because of 2 days of fever and productive cough. Amplification with universal coronavirus primers revealed a coronaviral sequence, and amplification of the complete genome showed that the virus was a novel group II human coronavirus only distantly related to HCoV-OC43.

In the 1960s many strains of human coronaviruses were cultured. Unfortunately, all except two (HCoV-229E and HCoV-OC43) were lost for further studies. It is possible that some of these were actually HCoV-NL63 or HCoV-HKU1 strains, but viral sequences of these strains are lacking so this will remain a mystery.

Clinical symptoms observed during infection by HCoV-NL63 and HCoV-HKU1

Nowadays, inoculation of volunteers to determine the spectrum of disease caused by a novel virus of uncertain pathogenicity is no longer ethically defensible. Therefore the only methods to investigate respiratory symptoms caused by a newly identified virus are through animal model studies and detailed epidemiological studies with appropriate controls. Unfortunately, there is no animal model available for the novel HCoV-NL63 and HCoV-HKU1 viruses. Even worse, not even a culture system is on hand for HCoV-HKU1. Therefore it is not possible to fulfill Koch's postulates for these viruses. The best method to unravel the relation between the viruses and disease is to determine whether there is a significant association with a disease.

HCoV-NL63 and HCoV-HKU1 have spread worldwide, with infections in Europe, North America, Australia and Asia [29–42]. The index patients in whom the viruses were first described suffered from severe LRTIs such as pneumonia and bronchiolitis. But the viruses can also be identified in patients with URTIs [8,31,43]. Furthermore, the high frequency of double infections is of note, especially in the HCoV-NL63 infected patients [26,29,30,44]. To obtain a clear picture of the symptoms observed in infected patients, only those persons without a second respiratory infection should be investigated in detail. In Table 1 and Table 2 the symptoms described for single HCoV-NL63 or HCoV-HKU1 infections are listed. Fever, cough and rhinorrhoea are frequently observed, and infection is often diagnosed in patients with an underlying disease. Most, if not all, patients that are hospitalized with HCoV-NL63 or HCoV-HKU1 infections are children,

adults with underlying disease or elderly [45]. Of the patients without an underlying disease only one HCoV-NL63-infected elderly person died from the respiratory disease [31]. Two HCoV-HKU1-infected adults died, but they had severe underlying illness (diabetes mellitus, old myocardial infarction and gastric lymphoma in one patient, and prostate carcinoma, cerebrovascular accident and diabetes mellitus in the other patient [46]). So, in general, infection by HCoV-NL63 and/or HCoV-HKU1 is not lethal. A large study of Hong Kong children with coronavirus and acute respiratory tract infection analysed the symptoms related to HCoV-HKU1 infection (Table 2). In these children febrile seizures were frequently observed (38%), and these occur less often in children infected by HCoV-OC43 (6%, $P<0.05$ [44]). Also, in single HCoV-NL63 infections febrile seizures occur quite often (18% in [47], 30% in [30]), but a feature typical of HCoV-NL63 infection is croup. Hong Kong studies and a Korean study reported high frequencies of HCoV-NL63 in children with croup (Table 1, [30,39,47]) (see also below: 'HCoV-NL63 infection and association with disease').

HCoV-NL63 infection and association with disease

Strictly speaking, an association with disease can only be determined when an appropriate control group without disease is included. Most studies of HCoV-NL63 and all studies of HCoV-HKU1 did not include a control group. For this reason, all symptoms described with infections should be taken with some caution, because it cannot be discounted that infections might occur that are not the causative agent for a disease. In total there are only three studies on HCoV-NL63 that included a control group.

As mentioned in the previous section, several studies observed the high incidence of croup among HCoV-NL63 infected children [30,39,47]. In one study HCoV-NL63 in croup and non-croup children was analysed [44]. The virus was present in 17% of the children with croup, compared with only 4% in the control group of children that suffered from non-croup illnesses like bronchiolitis and pneumonia ($P<0.0001$). Croup can be caused by several respiratory viruses, but in the literature the parainfluenzaviruses are regarded as the main causative agents for croup [48]. In the aforementioned study of croup children, HCoV-NL63 virus was detected even more frequently than the parainfluenzaviruses [44].

HCoV-NL63 has also been associated with Kawasaki disease [49]. Kawasaki disease is one of the most common forms of childhood vasculitis [50]. It presents with prolonged fever and a polymorphic exanthem, oropharyngeal erythema and bilateral conjunctivitis. A number of epidemiological and clinical observations

Table 1. Clinical symptoms and diagnosis in HCoV-NL63-positive patients without a second respiratory infection

			Symptoms									
Country	*n*	NL63 positive*	Fever	Cough	Coryza /rhino-rrhoea	Pharyngitis /sore throat /hoarseness	Bronchitis/ bronchiolitis	Pneu-monia	Croup	Underlying disease	Kawa-saki disease	Refer-ences
Netherlands	4	2.9	50	50	75	ND	0	0	0	100	0	[25]
Australia	10	2.1	70	70	60	50	50	0	10	30	0	[29]
Canada I	19	3.6	79	47	10	26	10	0	5	5	0	[31]
Japan†	3	2.5	100	100	100	33	100	0	33	0	0	[32]
Belgium	5	2.3	80	60	20	20	60‡	0	0	80	0	[33]
Hong Kong	12	2.6	18	73	64	27	0	0	27	27	0	[30]
France	18§	9.3	61	ND	39	22	33	5	0	5	0	[34]
Canada II	24	2.1	30	58	42	8	46	4	4	69	0	[43]
Japan II	5	1.2	100	80	80	0	20	0	0	60	0	[80]
Switzerland¶	4	7.3	100	100	100	75	ND#	ND#	ND#	0	0	[35]
Germany¶	20	5.2	40	ND	ND	ND	65	5	45	0	0	[44]
USA	79	8.8	48	64	61	ND**	ND**	ND**	0	52	1	[26,49]
Canada III	3	3.0	ND	100	100	ND	33	0	0	33	0	[56]
Korea¶	6	1.6	100	ND	ND	ND	0	33	50	0	0	[39]
Italy	8	1.1	14	43	57	ND	0	0	ND	ND	0	[40]
Hong Kong II	17	0.4	ND	ND	ND	ND	6	18	12	59	0	[47]
Sweden	11	6.0	27	27	54	18	18	0	0	9	0	[42]

Percentages of HCoV-NL63 patients with each symptom are given. ND (not determined) indicates that either it was uncertain whether the symptom was scored or it was not possible to calculate the percentage for only single NL63 infections *Total percentage of all samples tested showing HCoV-NL63 infections with single and double infections. Symptom statistics presented are of only single infections. ‡Selected for bronchiolitis. †Non-pneumonia LRTI. §Medical records were available for 18 of the 28 patients. ¶Selected for lower respiratory tract illness (LRTI). #No specification in LRTI was described. **Only individual clinical features, such as chest retractions, wheezing and abnormal chest radiography, were listed. From this the total number of patients with a specific diagnosis could not be determined.

suggested previously that an infectious agent might be the cause of Kawasaki disease (reviewed in [51]). Respiratory samples from 8/11 children (72.7%) with Kawasaki disease and from 1/22 control subjects (4.5%) were positive for HCoV-NL63 ($P<0.01$). This link between HCoV-NL63 and Kawasaki disease is intriguing, but at the same time is questioned. Several research groups screened for HCoV-NL63 in Kawasaki disease patients, but could not confirm the presumed association. Very low numbers of HCoV-NL63-positive respiratory samples were obtained from Kawasaki patients [52–55].

The third study that used a control group was conducted in Canada [56]. HCoV-NL63 was detected in nasopharyngeal aspirates from 3.0% of young children hospitalized for treatment of acute respiratory tract infections (12/396 children). However, the virus was also present in 1.7% of the samples obtained from asymptomatic control children (3/177). Because of the high frequency of HCoV-NL63 in the control group, the conclusion that HCoV-NL63 is not associated with respiratory tract illnesses seems defendable. However, the three control subjects that tested positive were perhaps not asymptomatic. These three children visited the hospital for myringotomy (n=2) and tonsillectomy (n=1). It is most likely that these surgical interventions were necessary because of chronic otitis media. Human coronavirus can be detected relatively frequently in children with otitis media (11%, [57]).

Sero-prevalence studies

The human coronaviruses are responsible for a high number of common cold cases each winter season [58]. This implies that during a lifetime almost every individual will experience an infection with these viruses, and consequently each will carry antibodies. The early literature on human coronaviruses demonstrated that antibodies to the viruses are frequently present [13]. However, in these antibody studies there was no discrimination between infections by viruses that belong to the same serotype (for example, HCoV-NL63 and HCoV-229E). A better understanding of the antibody response to the group I coronaviruses was provided by Hofmann *et al.* [59]. They used pseudotyping to measure specific neutralizing antibodies to HCoV-NL63 and HCoV-229E in human sera. Retroviral particles expressing the HCoV-NL63 spike protein or the HCoV-229E spike protein were compared in neutralization assays. From this study it could be concluded that virtually all sera obtained from adults were able to neutralize HCoV-NL63. A completely different result was obtained with 229E pseudovirions. HCoV-229E neutralization occurred only in a minority of the samples from healthy adults [59]. This indicates that HCoV-229E infections occur less frequently than HCoV-NL63 infections.

In the first publication on HCoV-HKU1 an ELISA-based antibody test was used to measure specific antibodies directed to the HKU1-nucleocapsid protein [9]. During the HCoV-HKU1 infection of the index patient a seroconversion for HCoV-HKU1 could be clearly observed. However, the antibody levels to HCoV-HKU1 in healthy adults are generally low, with only 2% of the healthy adults displaying a significant antibody titre directed to the nucleocapsid protein [9]. Unfortunately it is not possible to determine levels of neutralizing antibodies, because there is no culture system for HCoV-HKU1. A low frequency of HKU1 antibodies in adults might imply that HCoV-HKU1 has been recently introduced into humans via zoonotic transmission. However, the worldwide spread of the virus, its low mortality and its heterogeneity contradict this theory [36–38,60]. The situation might be similar to that of the group I coronaviruses. HCoV-NL63 infections occur more frequently than HCoV-229E [33,35,59], and the antibody levels to HCoV-NL63 are higher than the levels for HCoV-229E. Of the group II viruses, infection by HCoV-OC43 occurs more frequently than infection by HCoV-HKU1 [47], and, like the 229E–NL63 situation, the levels of

Table 2. Clinical symptoms and diagnosis in HCoV-HKU1-positive patients without a second respiratory infection

			Symptoms									
Country	*n*	HKU1-positive*	Fever	Cough	Coryza/rhinorrhoea	Pharyngitis/sore throat/hoarse ness	Bronchitis/bronchiolitis	Pneumonia	Croup	Underlying disease	Febrile seizures	Reference
Hong Kong	2	0.5	ND	ND	ND	ND	ND	100	0	50	0	[9]
Australia	9	3.1	ND	ND	ND	ND	ND	ND	ND	ND	ND	[36]
Hong Kong II†	10	2.4	80	70	10	20	0	100	0	80	0	[46]
France	5	4.4	60	0	20	0	0	0	0	60	20	[37]
USA	9	1.0	60	50	70	ND	10	20		50	0	[38]
Hong Kong III	13	0.3	ND	ND	ND	ND	8	8	0	62	38	[47]

Percentages of HCoV-HKU1 patients with each symptom are given. *Total percentage of all samples tested showing HCoV-HKU1 infections with single and double infections. Symptom statistics presented are of only single infections. †Only pneumonia.

antibodies to HCoV-OC43 in the human population might be higher than for HCoV-HKU1.

Treatment options

At present, there are no effective antiviral options available to treat infection by HCoV-229E, HCoV-OC43, HCoV-NL63 or HCoV-HKU1. Most current antivirals that are designed for coronaviruses target SARS-CoV and have not been tested for inhibition of the coronaviruses that are currently circulating in the human population. However, an effective antiviral treatment might be required for the coronavirus infection of children, elderly or patients with underlying illnesses. Several inhibitors are known to reduce *in vitro* replication of at least some coronaviruses, including HCoV-NL63 and/or HCoV-229E. These inhibitors act at various steps of the coronavirus replication cycle, including receptor binding, membrane fusion, transcription, replication and post-translational processing.

Inhibition of the fusion of the viral and cellular membranes is an attractive objective for therapy. Two heptad repeat regions, HR1 and HR2, are situated within the S2 part of the spike protein. Subsequent to binding of the virus to its receptor, the spike protein changes its conformation, forming a six-helix bundle containing 3 HR1s and 3 HR2s [61]. At the same time a viral fusion peptide is exposed which mediates membrane fusion between the virus membrane and the host cell membrane. Synthetic HR2 peptides are potent inhibitors of viral entry because an HR2 peptide interacts with the viral-HR1, blocking the formation of the natural six-helix HR1–HR2 bundle. As a result, membrane fusion is prevented. The inhibitory effect of synthetic HR2 peptides has been shown for retroviruses, paramyxoviruses and coronaviruses [62,63].

Inhibition of viral replication can also be achieved by degrading the viral genome using RNA interference (RNAi) [64]. *In vitro* experiments revealed that small interfering RNA (siRNA) molecules that target the HCoV-NL63 genome efficiently inhibit viral replication [63]. Experiments in animals have proven that virus-specific siRNAs administered intratracheally can be used to inhibit infection by respiratory syncytial virus (RSV), parainfluenza virus and SARS-CoV [65–67]. In the future, inhalation of a cocktail of siRNAs aimed at all different coronaviruses, or, preferably, all respiratory viruses, might provide an effective therapy to block viral replication in the lungs.

Several compounds can inhibit coronaviral replication at the level of transcription. Examples are the pyrimidine nucleoside analogues β-D-N^4-hydroxycytidine and 6-azauridine [63]. However, the exact mechanism by which these agents inhibit transcription during viral replication is unclear.

SARS-infected patients were often treated with ribavirin, but there is no evidence that it led to recovery. Instead, haemolytic anaemia, a side effect of this treatment, was observed in some studies [68]. For HCoV-NL63 and HCoV-229E an *in vitro* inhibitory effect of ribavirin was not observed [63,69].

Type I interferons (IFN) are part of the innate immune response and are produced early after viral infection. These interferons inhibit a wide range of viruses, including SARS-CoV and HCoV-229E [69–73]. Intranasal sprays of interferon given 1 day and 3 days after HCoV-229E challenge can protect volunteers from infection. However, longer dosage of interferon gives rise to nasal symptoms such as bloodstained discharge, which makes compliance difficult [74,75].

Intravenous immunoglobulin (IVIG) is successfully used to treat several diseases, mostly primary immune deficiencies and autoimmune neuromuscular disorders, but also respiratory diseases including RSV [76] and Kawasaki disease [77]. HCoV-NL63 can be inhibited, *in vitro*, by IVIG [63], but it is not known whether IVIG will also inhibit replication of other coronaviruses. During the SARS-CoV outbreak, IVIG was used to treat patients. It was thought that the immunomodulatory effect of IVIG might be of benefit during SARS infection. In general, the patients seemed to improve upon IVIG treatment, but more controlled trials are needed to obtain evidence of an effect for SARS [68].

Protease inhibitors act at the level of post-translational processing. The main protease (M^{pro}) of corona-viruses has a conserved substrate-recognition pocket and is therefore the perfect target for broad-spectrum antiviral drugs [78,79]. Yang *et al.* designed main protease inhibitors and measured the inhibitory capacity in an *in vitro* protease-activity assay. One potent inhibitor (N3) showed wide-spectrum inhibition of various M^{pro} enzymes, including the ones encoded by HCoV-229E, HCoV-NL63, HCoV-HKU1 and SARS-CoV [78].

Conclusions

Human coronaviruses are common cold viruses that can elicit a more serious respiratory disease in children, elderly and persons with underlying illness. This is true for the 'old' viruses HCoV-229E and HCoV-OC43, but uncertainties remain for HCoV-NL63 and HCoV-HKU1. Although it is clear that these new coronaviruses can be found in a significant percentage of URTIs and LRTIs, proof that these viruses cause common colds in normal adults is lacking. Screening for these viruses in subjects with common cold might provide the necessary evidence that these viruses are not that different from the 'old' viruses. Thus far, only HCoV-NL63 has been connected to a specific respiratory disease in children, namely croup. The suggested association between

HCoV-NL63 and Kawasaki disease remains doubtful. The link between HCoV-HKU1 and febrile seizure is interesting, but requires further investigation. To this end, children with and those without febrile seizure should be investigated. Only then can the necessary evidence as to whether an infection is significantly associated with a clinical symptom be supplied.

Acknowledgements

Lia van der Hoek is supported by VIDI grant 016.066.318 from Netherlands Organization for Scientific Research (NWO).

References

1. Drosten C, Gunther S, Preiser W, *et al*. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *N Engl J Med* 2003; **348:**1967–1976.
2. Ksiazek TG, Erdman D, Goldsmith CS, *et al*. A novel coronavirus associated with severe acute respiratory syndrome. *N Engl J Med* 2003; **348:**1953–1966.
3. Osterhaus AD, Fouchier RA, Kuiken T. The aetiology of SARS: Koch's postulates fulfilled. *Philos Trans R Soc Lond B Biol Sci* 2004; **359:**1081–1082.
4. Li W, Shi Z, Yu M, *et al*. Bats are natural reservoirs of SARS-like coronaviruses. *Science* 2005; **310:**676–679.
5. Lau SK, Woo PC, Li K, *et al*. Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats. *Proc Natl Acad Sci U S A* 2005; **102:**14040–14045.
6. Tyrrell DAJ, Bynoe ML. Cultivation of novel type of common-cold virus in organ cultures. *Br Med J* 1965; **1:**1467–1470.
7. Hamre D, Procknow JJ. A new virus isolated from the human respiratory tract. *Proc Soc Exp Biol Med* 1966; **121:**190–193.
8. van der Hoek L, Pyrc K, Jebbink MF, *et al*. Identification of a new human coronavirus. *Nat Med* 2004; **10:**368–373.
9. Woo PC, Lau SK, Chu CM, *et al*. Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. *J Virol* 2005; **79:**884–895.
10. Bradburne AF, Bynoe ML, Tyrrell DA. Effects of a "new" human respiratory virus in volunteers. *Br Med J* 1967; **3:**767–769.
11. Kendall EJ, Bynoe ML, Tyrrell DA. Virus isolations from common colds occurring in a residential school. *Br Med J* 1962; **5297:**82–86.
12. McIntosh K, Dees JH, Becker WB, Kapikian AZ, Chanock RM. Recovery in tracheal organ cultures of novel viruses from patients with respiratory disease. *Proc Natl Acad Sci U S A* 1967; **57:**933–940.
13. Bradburne AF, Somerset BA. Coronative antibody tires in sera of healthy adults and experimentally infected volunteers. *J Hyg (Lond)* 1972; **70:**235–244.
14. McIntosh K, Kapikian AZ, Hardison KA, Hartley JW, Chanock RM. Antigenic relationships among the coronaviruses of man and between human and animal coronaviruses. *J Immunol* 1969; **102:**1109–1118.
15. Hendley JO, Fishburne HB, Gwaltney JM Jr. Coronavirus infections in working adults. Eight-year study with 229E and OC43. *Am Rev Respir Dis* 1972; **105:**805–811.
16. McIntosh K, Chao RK, Krause HE, Wasil R, Mocega HE, Mufson MA. Coronavirus infection in acute lower respiratory tract disease of infants. *J Infect Dis* 1974; **130:**502–507.
17. Nicholson KG, Kent J, Hammersley V, Cancio E. Acute viral infections of upper respiratory tract in elderly people living in the community: comparative, prospective, population based study of disease burden. *BMJ* 1997; **315:**1060–1064.
18. van Elden LJ, van Loon AM, van Alphen F, *et al*. Frequent detection of human coronaviruses in clinical specimens from patients with respiratory tract infection by use of a novel real-time reverse-transcriptase polymerase chain reaction. *J Infect Dis* 2004; **189:**652–657.
19. Gerna G, Passarani N, Battaglia M, Revello MG, Torre D, Cereda PM. Coronaviruses and gastroenteritis: evidence of antigenic relatedness between human enteric coronavirus strains and human coronavirus OC43. *Microbiologica* 1984; **7:**315–322.
20. Fazakerley JK, Buchmeier MJ. Pathogenesis of virus-induced demyelination. *Adv Virus Res* 1993; **42:**249–324.
21. Burks JS, DeVald BL, Jankovsky LD, Gerdes JC. Two coronaviruses isolated from central nervous system tissue of two multiple sclerosis patients. *Science* 1980; **209:**933–934.
22. Murray RS, Brown B, Brian D, Cabirac GF. Detection of coronavirus RNA and antigen in multiple sclerosis brain. *Ann Neurol* 1992; **31:**525–533.
23. Dessau RB, Lisby G, Frederiksen JL. Coronaviruses in brain tissue from patients with multiple sclerosis. *Acta Neuropathol (Berl)* 2001; **101:**601–604.
24. Arbour N, Day R, Newcombe J, Talbot PJ. Neuroinvasion by human respiratory coronaviruses. *J Virol* 2000; **74:**8913–8921.
25. Fouchier RA, Hartwig NG, Bestebroer TM, *et al*. A previously undescribed coronavirus associated with respiratory disease in humans. *Proc Natl Acad Sci U S A* 2004; **101:**6212–6216.
26. Esper F, Weibel C, Ferguson D, Landry ML, Kahn JS. Evidence of a novel human coronavirus that is associated with respiratory tract disease in infants and young children. *J Infect Dis* 2005; **191:**492–498.
27. van der Hoek L, Berkhout B. Questions concerning the New Haven coronavirus. *J Infect Dis* 2005; **192:**350–351.
28. Lambert S, Mackay IM, Sloots TP, Nissen MD. Human coronavirus nomenclature. *Pediatr Infect Dis J* 2006; **25:**662.
29. Arden KE, Nissen MD, Sloots TP, Mackay IM. New human coronavirus, HCoV-NL63, associated with severe lower respiratory tract disease in Australia. *J Med Virol* 2005; **75:**455–462.
30. Chiu SS, Chan KH, Chu, KW, *et al*. Human coronavirus NL63 infection and other coronavirus infections in children hospitalized with acute respiratory disease in Hong Kong, China. *Clin Infect Dis* 2005; **40:**1721–1729.
31. Bastien N, Anderson K, Hart L, *et al*. Human coronavirus NL63 infection in Canada. *J Infect Dis* 2005; **191:**503–506.
32. Ebihara T, Endo R, Ma X, Ishiguro N, Kikuta H. Detection of human coronavirus NL63 in young children with bronchiolitis. *J Med Virol* 2005; **75:**463–465.
33. Moes E, Vijgen L, Keyaerts E, *et al*. A novel pancoronavirus RT-PCR assay: frequent detection of human coronavirus NL63 in children hospitalized with respiratory tract infections in Belgium. *BMC Infect Dis* 2005; **5:**6.
34. Vabret A, Mourez T, Dina J, *et al*. Human coronavirus NL63, France. *Emerg Infect Dis* 2005; **11:**1225–1229.
35. Kaiser L, Regamey N, Roiha H, Deffernez C, Frey U. Human coronavirus NL63 associated with lower respiratory tract symptoms in early life. *Pediatr Infect Dis J* 2005; **24:**1015–1017.
36. Sloots TP, McErlean P, Speicher DJ, Arden KE, Nissen MD, Mackay IM. Evidence of human coronavirus HKU1 and human bocavirus in Australian children. *J Clin Virol* 2006; **35:**99–102.
37. Vabret A, Dina J, Gouarin S, Petitjean J, Corbet S, Freymuth F. Detection of the new human coronavirus HKU1: a report of 6 cases. *Clin Infect Dis* 2006; **42:**634–639.
38. Esper F, Weibel C, Ferguson D, Landry ML, Kahn JS. Coronavirus HKU1 infection in the United States. *Emerg Infect Dis* 2006; **12:**775–779.
39. Choi EH, Lee HJ, Kim SJ, *et al*. The association of newly identified respiratory viruses with lower respiratory tract infections in Korean children, 2000–2005. *Clin Infect Dis* 2006; **43:**585–592.

40. Gerna G, Campanini G, Rovida F, *et al*. Genetic variability of human coronavirus OC43-, 229E-, and NL63-like strains and their association with lower respiratory tract infections of hospitalized infants and immunocompromised patients. *J Med Virol* 2006; **78:**938–949.
41. Garbino J, Crespo S, Aubert JD, *et al*. A prospective hospital-based study of the clinical impact of non-severe acute respiratory syndrome (Non-SARS)-related human coronavirus infection. *Clin Infect Dis* 2006; **43:**1009–1015.
42. Koetz A, Nilsson P, Linden M, van der Hoek L, Ripa T. Detection of human coronavirus NL63, human metapneumovirus and respiratory syncytial virus in children with respiratory tract infections in south-west Sweden. *Clin Microbiol Infect* 2006; **12:**1089–1096.
43. Bastien N, Robinson JL, Tse A, Lee BE, Hart L, Li Y. Human coronavirus NL-63 infections in children: a 1-year study. *J Clin Microbiol* 2005; **43:**4567–4573.
44. van der Hoek L, Sure K, Ihorst G, *et al*. Croup is associated with the novel coronavirus NL63. *PLoS Med* 2005; **2:**e240.
45. Hayden FG. Respiratory viral threats. *Curr Opin Infect Dis* 2006; **19:**169–178.
46. Woo PC, Lau SK, Tsoi HW, *et al*. Clinical and molecular epidemiological features of coronavirus HKU1-associated community-acquired pneumonia. *J Infect Dis* 2005; **192:**1898–1907.
47. Lau SK, Woo PC, Yip CC, *et al*. Coronavirus HKU1 and other coronavirus infections in Hong Kong. *J Clin Microbiol* 2006; **44:**2063–2071.
48. Denny FW, Murphy TF, Clyde WA Jr, Collier AM, Henderson FW. Croup: an 11-year study in a pediatric practice. *Pediatrics* 1983; **71:**871–876.
49. Esper F, Shapiro ED, Weibel C, Ferguson D, Landry ML, Kahn JS. Association between a novel human coronavirus and Kawasaki Disease. *J Infect Dis* 2005; **191:**499–502.
50. Burns JC, Glode MP. Kawasaki syndrome. *Lancet* 2004; **364:**533–544.
51. Burgner D, Harnden A. Kawasaki disease: what is the epidemiology telling us about the etiology? *Int J Infect Dis* 2005; **9:**185–194.
52. Ebihara T, Endo R, Ma X, Ishiguro N, Kikuta H. Lack of association between New Haven coronavirus and Kawasaki disease. *J Infect Dis* 2005; **192:**351–352.
53. Belay ED, Erdman DD, Anderson LJ, *et al*. Kawasaki disease and human coronavirus. *J Infect Dis* 2005; **192:**352–353.
54. Shimizu C, Shike H, Baker SC, *et al*. Human coronavirus NL63 is not detected in the respiratory tracts of children with acute Kawasaki Disease. *J Infect Dis* 2005; **192:**1767–1771.
55. Chang LY, Chiang BL, Kao CL, *et al*. Lack of association between Infection with a Novel Human Coronavirus (HCoV), HCoV-NH, and Kawasaki Disease in Taiwan. *J Infect Dis* 2006; **193:**283–286.
56. Boivin G, Baz M, Cote S, *et al*. Infections by human coronavirus-NL in hospitalized children. *Pediatr Infect Dis J* 2005; **24:**1045–1048.
57. Bulut Y, Guven M, Otlu B, *et al*. Acute otitis media and respiratory viruses. *Eur J Pediatr* 2007; **166:**223–228.
58. McIntosh K (1996). Coronaviruses. In *Fields Virology,* 3rd edn; pp. 1095–1103 Edited by BN Fields, DM Knipe, PM Howley, *et al*. Philadelphia: Lippincott-Raven Publishers.
59. Hofmann H, Pyrc K, van der Hoek L, Geier M, Berkhout B, Pohlmann S. Human coronavirus NL63 employs the severe acute respiratory syndrome coronavirus receptor for cellular entry. *Proc Natl Acad Sci U S A* 2005; **102:**7988–7993.
60. Woo PC, Lau SK, Yip CC, *et al*. Comparative analysis of 22 coronavirus HKU1 genomes reveals a novel genotype and evidence of natural recombination in coronavirus HKU1. *J Virol* 2006; **80:**7136–7145.
61. Bosch BJ, van der Zee R, de Haan CA, Rottier PJ. The coronavirus spike protein is a class I virus fusion protein: structural and functional characterization of the fusion core complex. *J Virol* 2003; **77:**8801–8811.
62. Bosch BJ, Martina BE, van der Zee R, *et al*. Severe acute respiratory syndrome coronavirus (SARS-CoV) infection inhibition using spike protein heptad repeat-derived peptides. *Proc Natl Acad Sci U S A* 2004; **101:**8455–8460.
63. Pyrc K, Bosch BJ, Berkhout B, *et al*. Inhibition of HCoV-NL63 infection at early stages of the replication cycle. *Antimicrob Agents Chemoth* 2006; **50:**2000–2008.
64. Haasnoot PCJ, Cupac D, Berkhout B. Inhibition of virus replication by RNA interference. *J Biomed Sc* 2003; **10:**607–616.
65. Bitko V, Musiyenko A, Shulyayeva O, Barik S. Inhibition of respiratory viruses by nasally administered siRNA. *Nat Med* 2005; **11:**50–55.
66. Li BJ, Tang Q, Cheng D, *et al*. Using siRNA in prophylactic and therapeutic regimens against SARS coronavirus in Rhesus macaque. *Nat Med* 2005; **11:**944–951.
67. Zhang W, Yang H, Kong X, *et al*. Inhibition of respiratory syncytial virus infection with intranasal siRNA nanoparticles targeting the viral NS1 gene. *Nat Med* 2005; **11:**56–62.
68. Stockman LJ, Bellamy R, Garner P. SARS: Systematic Review of Treatment Effects. *PLoS Med* 2006; **3:**e343.
69. Hertzig T, Scandella E, Schelle B, *et al*. Rapid identification of coronavirus replicase inhibitors using a selectable replicon RNA. *J Gen Virol* 2004; **85:**1717–1725.
70. Cinatl J, Morgenstern B, Bauer G, Chandra P, Rabenau H, Doerr HW. Treatment of SARS with human interferons. *Lancet* 2003; **362:**293–294.
71. Stroher U, DiCaro A, Li Y, *et al*. Severe acute respiratory syndrome-related coronavirus is inhibited by interferon-α. *J Infect Dis* 2004; **189:**1164–1167.
72. Tan EL, Ooi EE, Lin CY, *et al*. Inhibition of SARS coronavirus infection in vitro with clinically approved antiviral drugs. *Emerg Infect Dis* 2004; **10:**581–586.
73. Haagmans BL, Kuiken T, Martina BE, *et al*. Pegylated interferon-α protects type 1 pneumocytes against SARS coronavirus infection in macaques. *Nat Med* 2004; **10:**290–293.
74. Tyrrell DA. The efficacy and tolerance of intranasal interferons: studies at the Common Cold Unit. *J Antimicrob Chemother* 1986; **18 Suppl B:**153–156.
75. Jefferson TO, Tyrrell D. Antivirals for the common cold. *Cochrane Database Syst Rev* 2001; CD002743.
76. Hemming VG, Rodriguez W, Kim HW, *et al*. Intravenous immunoglobulin treatment of respiratory syncytial virus infections in infants and young children. *Antimicrob Agents Chemother* 1987; **31:**1882–1886.
77. Stiehm ER, Ashida E, Kim KS, Winston DJ, Haas A, Gale RP. Intravenous immunoglobulins as therapeutic agents. *Ann Intern Med* 1987; **107:**367–382.
78. Yang H, Xie W, Xue X, *et al*. Design of wide-spectrum inhibitors targeting coronavirus main proteases. *PLoS Biol* 2005; **3:**e324.
79. Blanchard JE, Elowe NH, Huitema C, *et al*. High-throughput screening identifies inhibitors of the SARS coronavirus main proteinase. *Chem Biol* 2004; **11:**1445–1453.
80. Suzuki A, Okamoto M, Ohmi A, Watanabe O, Miyabayashi S, Nishimura H. Detection of human coronavirus-NL63 in children in Japan. *Pediatr Infect Dis J* 2005; **24:**645–646.

Accepted for publication 14 February 2007