Questions concerning the new haven coronavirus: letter

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Questions Concerning the New Haven Coronavirus

To the Editor—Esper et al. present the discovery of a novel human coronavirus (HCoV) in young children and infants with respiratory tract disease in New Haven: HCoV-NH [1]. However, they also mention that the virus is very similar to HCoV-NL63, a virus that was identified previously in Amsterdam, The Netherlands [2]. Despite this, the 2 studies by Esper et al. [1, 3] and an Editorial Commentary by McIntosh [4] avoid usage of the name “HCoV-NL63” while repeatedly claiming the discovery of a novel virus.

To judge whether HCoV-NH is really a novel HCoV, a comparative analysis of HCoV-NH with a number of different features of established HCoVs should be performed. Examining the relatedness of genome sequences is one facet of such an analysis. Unfortunately, limited data on the genome sequence for HCoV-NH are available, but inspection of a 126-bp fragment clearly shows that all HCoV-NH isolates cluster together with the HCoV-NL63 Amsterdam-1 strain (figure 3 in [1]). This result strongly suggests that the viruses found by Esper et al. are New Haven isolates of HCoV-NL63. Moreover, the actual nucleotide difference between the New Haven isolates (GenBank accession nos. AY870943–AY871008) and the HCoV-NL63 isolate Amsterdam-1 (GenBank accession no. NC_005831) is 0%–6%. This degree of difference falls well within the range of genetic variation observed among different HCoV-NL63 isolates from Amsterdam [2]. We reported the presence of distinct HCoV-NL63 variants that apparently are cocirculating, as has been confirmed recently by Arden et al. [5] and Bastien et al. [6].

Esper et al. do not seem to dispute that HCoV-NH is very similar to HCoV-NL63. What then made them decide to claim the identification of a novel virus? The only argument mentioned is that the research project was initiated before the first article on HCoV-NL63 was published. Is that how it works in science? No—only the first report can claim a novel scientific finding. In fact, for HCoV-NL63, Esper et al.’s is the third article that claims its discovery. The identification of HCoV-NL63 was first announced in an article in Nature Medicine (which was published electronically on 21 March 2004 [2]), and an article by Fouchier et al. in the Proceedings of the National Academy of Sciences of the United States of America described the same virus [7]. Esper et al. submitted their manuscript to the Journal of Infectious Diseases on 7 September 2004, about 6 months after the initial description of HCoV-NL63 was published. Other studies on HCoV-NL63 were submitted for publication in 2004 (and some were published earlier than the one by Esper et al.), but all appropriately acknowledged the discovery of HCoV-NL63 and used its nomenclature, and none claimed the discovery of a novel virus [5–9]. In fact, the issue of the Journal of Infectious Diseases in which the study by Esper et al. was published also contains a survey on the prevalence of HCoV-NL63—and indeed, it appropriately names the virus—in Canada [6].

The Esper et al., Fouchier et al., and Arden et al. articles claim in their titles that HCoV-NL63 is associated with respiratory tract disease [1, 5, 7]. However, this conclusion can be drawn only when an appropriate patient control group without respiratory tract disease is included. These studies did not include such a control group, and it is therefore premature to conclude that HCoV-NL63 is associated with disease. The suggestion may be strong, but much work remains ahead of us to accurately define the spectrum of respiratory tract disease caused by HCoV-NL63. The study by Esper et al. on the association between HCoV-NH and Kawasaki disease did include the proper control group, and this finding is very intriguing indeed [3].

The detection of HCoV-NL63 in The Netherlands, Canada, Australia, Japan, and the United States convincingly demonstrates the worldwide distribution of HCoV-NL63. Proposing a different name for basically the same virus each time it is “rediscovered” needlessly complicates the HCoV literature.

Lia van der Hoek and Ben Berkhout
Department of Human Retrovirology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

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Lack of Association between New Haven Coronavirus and Kawasaki Disease

To the Editor—The new human coronavirus NL63 (HCoV-NL63) was discovered by van der Hoek et al. [1] and Fouchier et al. [2]. HCoV-NL63 has been shown to cause respiratory tract disease in young children [3, 4]. Esper et al. have reported a novel HCoV designated the “New Haven coronavirus” (HCoV-NH) that has been shown by sequence analysis to be very similar to HCoV-NL63 [5]. Esper et al. also reported that HCoV-NH was detected by reverse-transcription polymerase chain reaction (RT-PCR) in 8 (72.7%) of 11 respiratory tract samples from children with Kawasaki disease (KD) and in 1 (4.5%) of 22 age-matched samples from control subjects [6]. On the basis of these data, they suggested that HCoV-NH infection was associated with KD. To further investigate whether HCoV-NH disease is associated with KD, we performed a retrospective study.

From October 2002 to May 2003, 19 nasopharyngeal swab samples were collected from 19 children who fulfilled the criteria for KD and who were treated at Tenshi Hospital in Sapporo, Japan. All of the samples were collected after informed consent was obtained from the children’s parents. All of the samples were obtained within 7 days of the onset of illness. The mean age of the children with KD was 22.6 months (range, 4 months–5 years). We used as controls 208 nasopharyngeal swab samples that were collected from children with diagnoses of respiratory tract disease who were admitted to hospitals in Sapporo, Japan, during the same period. All of these samples were examined after the possibility of infection with human respiratory syncytial virus or influenza A or B was excluded by rapid antigen-detection tests. The mean age of the children with respiratory tract disease was 21.6 months (range, 4 months–5 years). After extraction of total RNA and synthesis of cDNA, we performed RT-PCR to detect the HCoV-NH genome, as described by Esper et al. [6]. The primer set and the PCR conditions in our PCR assay were the same as those used in their PCR assays. Sequencing of the PCR products was also performed to confirm the presence of HCoV-NH.

Although RNA sequences of HCoV-NH were detected in samples from 5 (2.4%) of the 208 control children with respiratory tract disease, we could not detect any RNA sequences of HCoV-NH in 19 samples from children with KD (table 1). On the basis of these data, we have some reservations about the findings described by Esper et al. [6]. They collected respiratory tract swab samples from children with KD as part of an ongoing epidemiological investigation of respiratory tract viruses. We collected respiratory tract swab samples from all of the patients with KD, regardless of the presence of respiratory tract symptoms, who were treated at Tenshi Hospital from October 2002 to May 2003. Because no RNA sequences of HCoV-NH were detected in samples from 19 patients with KD in our study, there is a possibility that Esper et al. tested samples from patients with KD who had respiratory tract symptoms. Our results suggest that Esper et al.’s results may be coincidental and that HCoV-NH does not play a dominant role in the etiology or pathogenesis of KD in Japan.

Table 1. Detection of New Haven coronavirus (HCoV-NH) in children with Kawasaki disease (KD) and in children with respiratory tract disease (RTDs).

<table>
<thead>
<tr>
<th>Date</th>
<th>Children with KD</th>
<th>Children with RTD</th>
</tr>
</thead>
<tbody>
<tr>
<td>October 2002</td>
<td>0/4</td>
<td>0/12</td>
</tr>
<tr>
<td>November 2002</td>
<td>0/4</td>
<td>0/27</td>
</tr>
<tr>
<td>December 2002</td>
<td>0/1</td>
<td>0/20</td>
</tr>
<tr>
<td>January 2003</td>
<td>0/2</td>
<td>1/20</td>
</tr>
<tr>
<td>February 2003</td>
<td>0/2</td>
<td>1/24</td>
</tr>
<tr>
<td>March 2003</td>
<td>0/3</td>
<td>3/26</td>
</tr>
<tr>
<td>April 2003</td>
<td>0/0</td>
<td>0/29</td>
</tr>
<tr>
<td>May 2003</td>
<td>0/3</td>
<td>0/50</td>
</tr>
<tr>
<td>Total</td>
<td>0/19 (0.0%)</td>
<td>5/208 (2.4%)</td>
</tr>
</tbody>
</table>

References
Kawasaki Disease and Human Coronavirus

To the Editor—Esper et al. [1] recently reported the possible causal association between a novel human coronavirus (HCoV) and Kawasaki disease (KD). They reported that respiratory secretions from 72.7% of 11 patients with KD but from only 4.5% of 22 age-matched control subjects tested positive for an HCoV designated by Esper et al. as the "New Haven coronavirus" (HCoV-NH). This virus was reported to be closely related to HCoV-NL and HCoV-NL63, which were identified by 2 independent groups from The Netherlands [2, 3].

To determine if HCoV might be consistently associated with KD, we analyzed nasopharyngeal and oropharyngeal swab samples collected in 1999 as part of an etiologic investigation of KD in San Diego. The 1999 investigation focused on exploring the possible causal link between KD and Chlamydia pneumoniae, and the details of the investigation and case-control design are described by Schrag et al. [4]. After the 1999 investigation was completed, the pharyngeal swab samples were stored at −70°C. Pharyngeal swab samples were available for analysis from 10 patients with KD and from 6 control subjects. The patients, who had onset of KD between 9 February and 20 March 1999, had a median age of 3.6 years (range, 0.6–8.6 years). The patients met the epidemiologic case definition for KD: they had fever lasting for ≥5 days and had at least 4 of the 5 clinical features of KD [4]. The median age for the control subjects was 3.3 years (range, 1.3–8.3 years). Pharyngeal swab samples were obtained within 10 days of the onset of illness in 6 of the patients with KD and on days 11, 15, 16, and 37 after the onset of illness in the remaining 4 patients.

All pharyngeal swab samples from the patients with KD and from the control subjects tested negative for HCoV by use of 2 different primer sets. Nucleic acid was extracted from 200 μL of the pharyngeal swab samples by use of the automated NucliSens Extractor (bioMérieux). Twenty-five-microliter reactions containing 5 μL of the extracted nucleic acid were prepared with the 1-step Access RT-PCR System (Promega). The first primer set used for amplification was an HCoV-NH/HCoV-NL63–specific primer described by Esper et al. [1] that had the following modification: a single nucleotide degeneracy was introduced into the sense-strand primer, 5′-GGGCTGAGGGTGTTG-3′, to accommodate a sequence variation among published sequences of HCoV-NH/HCoV-NL63 strains (the underlining indicates the modification). The amplification program consisted of a reverse-transcription (RT) step of 45 min at 45°C, 2 min at 94°C, to denature the reverse transcriptase; 40 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C; and 10 min at 72°C, for final amplicon extension. The second RT–polymerase chain reaction (PCR) primer set had broadly reactive primers designed to target highly conserved regions of the HCoV RNA polymerase gene: sense-strand primer 5′-GGTTGGGATTATCC-3′ and antisense strand primer 5′-TATAACACACCAACCCYTCATCA-3′. Amplification reactions were performed as described above, and the following program settings were used: an RT step of 45 min at 45°C and 2 min at 94°C, to denature the reverse transcriptase; 40 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C; and 10 min at 72°C, for final amplicon extension.

The sense-strand primers for both sets were labeled with Cy5 fluorescent dye at the 5′ end to facilitate amplicon detection (the predicted sizes for the first and second primer sets were 215 and 454 bp, respectively) using the CEQ 8000 Genetic Analysis System (Beckman Coulter). Assays were performed using standard viral nucleic acid extracts (HCoV 229E and OC43) and nuclease-free water for positive and negative controls, respectively. All pharyngeal swab samples tested positive by RT-PCR for human glyceraldehyde-3-phosphate dehydrogenase enzyme, which indicated that there was adequate recovery of RNA from the samples and that RT-PCR inhibitors were absent.

Our findings do not support those of Esper et al. [1]. Methodologic differences in the type and timing of sample collection; in sample handling, storage, and processing; and in the selection of case patients and control subjects may explain our different findings. Alternatively, different etiologic agents could have been associated with KD in the 2 study populations. Further studies that include serologic testing and prospectively collected high-quality pharyngeal swab samples may be needed to determine the role, if any, that HCoVs play in the etiology of KD.

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In fact, the International Committee on nomenclature and categorization of viruses is only one of many criteria used to differentiate between viral species [5]. Undoubtedly, this will continue to be an issue as new viral pathogens are identified.

We disagree with van der Hoek and Berkhout that the naming of HCoV-NH “needlessly complicates the HCoV literature” [4]. Rather, we believe that the identification of HCoV-NL63 and HCoV-NL by cell culture and genome amplification techniques and the identification of HCoV-NH by use of molecular probes exemplify the recent advances in methods to identify previously unrecognized viruses. As the full genomes of these viruses are described, the clarification of the nomenclature will follow.

We believe it is important to attempt to replicate in different populations our finding of the association between HCoV-NH infection and Kawasaki disease, and we appreciate the letters from Ebihara et al. [7] and Belay et al. [8]. We would be interested to know whether proper controls were included to demonstrate the integrity of the RNA that was used in the amplification assays by Ebihara et al. We agree with Belay et al. that the timing of sample collection and the type of sample screened (nasal vs. pharyngeal) may explain the discrepancy in results between their study and ours. Furthermore, more than one epidemiological agent may be linked to Kawasaki disease. Nonetheless, we are intrigued by the study by Graf that suggests the presence of a peptide, corresponding to the spike protein of HCoV-NL63, in tissue from individuals with Kawasaki disease [9]. These findings further support the association between HCoV-NH and Kawasaki disease.

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Reply to van der Hoek and Berkhout

To the Editor—In my Editorial Commentary [1], I certainly did not intend to detract from the novelty and importance of the findings by van der Hoek et al. [2] in early 2004. As I pointed out clearly, Esper et al. [3] were unaware of van der Hoek et al.’s discovery of the Netherlands strain of human coronavirus (HCoV-NL63) when they designed their polymerase chain reaction (PCR) and apparently also when they first found the HCoV they designated...
the “New Haven coronavirus” (HCoV-NH). I described the methodology used by Esper et al. for finding the virus as “logical, intelligent, and highly original” (p. 489)—as was, in fact, the different methodology used by van der Hoek et al.—and the development of this methodology seemed to me at least as notable an accomplishment as the discovery of a “new” virus. In my Editorial Commentary, I also stated that HCoV-NH appeared to be similar to, and quite possibly (although not certainly) the same as, HCoV-NL63. The published sequence of HCoV-NH, which covered only a portion of a single gene from this very large genome, did not seem to be adequate to make a final decision on this point, however.

Regardless of whether HCoV-NH turns out to be, from a taxonomic viewpoint, the same as HCoV-NL63, the findings by Esper et al. are exciting and extend our knowledge of respiratory HCoVs, particularly in relation to pediatric disease. In addition, broader application of their PCR methodology offers to us the hope that we will find additional members of this family of viruses.

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