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References


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Coronaviruses in Children, Greece

To the Editor: Two recently detected human coronaviruses (HCoVs), NL63 and HKU1, increased the number of coronaviruses known to infect humans to 5 (1–3). HCoV-229E and HCoV-NL63 belong to antigenic group 1, HCoV-OC43 and HCoV-HKU1 belong to antigenic group 2, and severe acute respiratory syndrome (SARS)–associated coronavirus (SARS-CoV) is most closely related to group 2 coronaviruses. In 2005, an optimized pancoronavirus reverse transcription–PCR assay was used to explore the incidence of HCoV-NL63 infection in children in Belgium who had a diagnosis of respiratory tract infection (4). We report the results of an epidemiologic study that used a universal coronavirus RT-PCR assay to detect coronaviruses among children in Greece with acute respiratory tract infections.

We tested throat swab specimens obtained from children hospitalized in Greece during June 2003 through May 2004 (200 children 2 months to 14 years of age, mean 4.09 years) and during December 2005 through March 2006 (44 children 1.6–8.5 years of age, mean 5.05). Specimens were obtained the first day of each child’s hospitalization, and all specimens were included in the study, regardless whether other respiratory microorganisms were detected.

The 25-μL reaction contained 200 μM dNTPs, 0.2 μM primer PC2S2 (equimolar mixture of 5′-TTATGGGTTGGGATTATC-3′ and 5′-TGATGGGATGGGACTATC-3′), 0.8 μM primer PC2As1 (5′-TCATCAGAAAGATCAGTC-3′), 1 μL of enzyme mix from the QIAGEN OneStep RT-PCR Kit (QIAGEN GmbH, Hilden, Germany), and 5 μL of RNA. The initial 30-min reverse transcription step at 48°C was followed by 10 cycles of 20 sec at 94°C, 30 sec at 62°C with a decrease of 1°C per cycle, 40 sec at 72°C; 40 cycles of 20 sec at 94°C, 30 sec at 52°C, 40 sec at 72°C; and a final extension step at 72°C for 10 min. To determine the sensitivity after optimization, we tested quantified RNA in vitro transcripts that included the natural primer binding sites of the respective coronavirus genomes. Sensitivities for SARS-CoV, HCoV-OC43, HCoV-229E, and HCoV-NL63 were 61.0, 800.0, 8.2, and 82.3 nominal RNA copies per assay, respectively. A separate test was not done for HCoV-HKU1 because it had the same primer binding sites as HCoV-OC43.

A phylogenetic tree based on a 400-bp genome fragment of the polymerase gene was constructed (online Appendix Figure, available from www.cdc.gov/EID/13/6/947-appG.htm).

Of 200 samples collected in 2003–2004, 5 (2.5%) were positive for coronaviruses (2 each for HCoV-NL63 and HCoV-229E and 1 for HCoV-OC43), and of 44 samples collected in 2005–2006, 2 (4.5%) were positive for coronaviruses (1 for HCoV-229E and 1 for HCoV-OC43) (GenBank accession nos. EF103180–EF103184, EF394298, and EF394299). CoV-HKU1 was not detected.

The amplified genome region is one of the most conserved regions of the coronavirus genome. However, sequences for HCoV-NL63 strains isolated in Greece are genetically closer to the sequence for a strain (AY567487) isolated in Amsterdam in 2003 (1) than to a strain (AY518894) from a specimen collected in Rotterdam in 1988 (2) (0.6% vs. 1.1% nucleotide divergence). Sequences for HCoV-229E and HCoV-OC43 strains isolated in Greece differ from sequences for strains isolated elsewhere by 0.5%–1.7%.

The HCoV-NL63–positive specimens in our study were obtained from a 9- and a 14-month-old child during winter 2003–2004; no cases were identified during 2005–2006. Specimens positive for HCoV-229E and HCoV-OC43 were detected during both study
periods (Table). HCoV-OC43 affected children with a mean age of 3.1 years (median, 1.4 years), and HCoV-229E affected children with a mean (and median) age of 5.5 years. However, no general conclusions can be drawn from these data because number of cases is too few.

None of the patients in Greece had an underlying disease, and all recovered completely. Patients infected with HCoV-229E had been hospitalized for upper respiratory tract infections, and those with HCoV-OC43 had lower respiratory tract infections; all cases were mild. Both children infected with HCoV-NL63 had symptoms of lower respiratory tract infections: 1 child had severe pneumonia and was hospitalized for 12 days, while the other had a mild course of bronchiolitis.

HCoV-NL63 was first identified in Amsterdam, the Netherlands, by van der Hoek et al. (1) from a nasopharyngeal specimen obtained in 2003 from a 7-month-old child with bronchiolitis, conjunctivitis, and fever. One month later, Fouchier et al. (2) reported the characterization of the same virus isolated from a specimen collected in 1988. The specimen had been obtained from an 8-month-old child with pneumonia in Rotterdam, the Netherlands. Later, HCoV-NL63 was detected in 2.5% of bronchiolitis patients <2 years of age in Japan (4) and in most children hospitalized with bronchiolitis in Australia and Canada (6,7).

Coinfection with HCoVs and other respiratory viruses is frequently observed and is associated with severe clinical syndromes, especially in infants and young children (6,8). Coinfection was observed in 3 of the 7 HCoV-positive patients in our study. The 3 patients were infected with HCoV-OC43 or HCoV-229E; coinfection with respiratory syncytial virus was found in 2 patients, and coinfection with Mycoplasma pneumoniae was found in 1 patient. It was not possible to determine the role of the HCoVs in these coinfections. In addition, because coronaviruses can be detected even 3 weeks after an acute episode, some cases of coinfection might represent former rather than current HCoV infection (9).

In conclusion, we detected 3 types of HCoVs in Greece: 229E, OC43, and NL63. This finding provides initial insight into the epidemiologic features of coronaviruses in Greece. Further studies are needed to find the exact clinical effect of these HCoVs in humans and to elucidate the epidemiology of coronaviruses worldwide.

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References


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### Table: Epidemiologic and laboratory data for patients with coronavirus infection, Greece*

<table>
<thead>
<tr>
<th>Specimen no., HCoV strain</th>
<th>Age, sex</th>
<th>Sample date</th>
<th>Symptoms</th>
<th>WBC (cells/ mm³)</th>
<th>Granulocytes, %</th>
<th>ESR (mm/h)</th>
<th>Days in hospital</th>
<th>Coinfection</th>
</tr>
</thead>
<tbody>
<tr>
<td>10/03, 229E</td>
<td>3 y, F</td>
<td>Jun 3, 2003</td>
<td>Fever (39°C), cough, pharyngitis</td>
<td>10,400</td>
<td>87</td>
<td>40</td>
<td>3</td>
<td>RSV</td>
</tr>
<tr>
<td>16/03, 229E</td>
<td>8 y, M</td>
<td>Jun 14, 2003</td>
<td>Fever (41°C), headache, rhinitis, sinusitis</td>
<td>18,900</td>
<td>86.4</td>
<td>30</td>
<td>4</td>
<td>ND</td>
</tr>
<tr>
<td>109/03, NL63</td>
<td>14 mo, F</td>
<td>Nov 30, 2003</td>
<td>Fever (39°C), cough, severe pneumonia</td>
<td>18,700</td>
<td>44.0</td>
<td>85</td>
<td>12</td>
<td>ND</td>
</tr>
<tr>
<td>173/04, NL63</td>
<td>10 mo, M</td>
<td>Feb 10, 2004</td>
<td>Fever (38.5°C), cough, rhinitis, tachypnea, bronchiolitis</td>
<td>7,100</td>
<td>57.9</td>
<td>55</td>
<td>3</td>
<td>ND</td>
</tr>
<tr>
<td>185/04, OC43</td>
<td>17 mo, F</td>
<td>Feb 25, 2004</td>
<td>Pharyngitis, rhinitis, respiratory distress, bronchiolitis</td>
<td>10,100</td>
<td>63.2</td>
<td>30</td>
<td>2</td>
<td>ND</td>
</tr>
<tr>
<td>12A/06, OC43</td>
<td>6 mo, F</td>
<td>Jan 11, 2006</td>
<td>Fever (38.8°C), cough, tachypnea, bronchiolitis</td>
<td>19,950</td>
<td>80.3</td>
<td>35</td>
<td>6</td>
<td>RSV</td>
</tr>
<tr>
<td>14A/06, 229E</td>
<td>7.5 y, M</td>
<td>Feb 13, 2006</td>
<td>Fever (40.5°C), cough, rhinitis</td>
<td>20,600</td>
<td>83.1</td>
<td>98</td>
<td>4</td>
<td>Mycoplasma pneumoniae</td>
</tr>
</tbody>
</table>

*HCoV, human coronavirus; WBC, white blood cell count; ESR, erythrocyte sedimentation rate; RSV, respiratory syncytial virus; ND, not detected.


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**Bartonella DNA in Loggerhead Sea Turtles**

To the Editor: *Bartonella* are fastidious, aerobic, gram-negative, facultative, intracellular bacteria that infect erythrocytes, erythroblasts, endothelial cells, monocytes, and dendritic cells, and are transmitted by arthropod vectors or by animal scratches or bites (1–6). Currently, 20 species or subspecies of *Bartonella* have been characterized, of which 8 are known zoonotic pathogens (7). *B. henselae* has been recently identified from canine blood (8) and from harbor porpoises (9). Pathogenic bacteria are an important threat in terrestrial and marine environments, and in the case of *B. henselae*, reservoir hosts may be more diverse than currently recognized.

The purpose of this study was to determine whether sea turtles are infected with *Bartonella* spp. Blood samples were obtained from 29 free-ranging and 8 captive, rehabilitating loggerhead sea turtles (*Caretta caretta*) from North Carolina coastal waters. Reptilian erythrocytes are nucleated, and commercial lysis methods were unsuccessful for these samples, while amplicons were obtained from 16S-23S ITS PCR–positive samples. The 3 Pap31 amplicons successfully sequenced confirmed *B. henselae* infection. Sequences obtained from 1 sample matched *B. henselae* strains H1-like, the *B. henselae* SA2-like strain, and *B. vinsonii* subs. *berkhoffii* genotypes II and IV, which suggests that this turtle was co-infected with multiple *Bartonella* spp. and strains. Three other samples yielded amplicons 99%–100% identical with *B. henselae* strain SA2, and 3 yielded sequences most similar to *B. vinsonii* subspecies *berkhoffii* genotypes II and IV. Two samples contained an ITS region sequence most similar to *B. henselae* SA2, but with a 15-bp deletion beginning 617 bases downstream from the 16S rRNA gene. Whether these ITS sequence differences represent distinct strains or nonrandom translocation events is uncertain.

Four sea turtle blood samples contained partial ITS sequences most similar to *B. vinsonii* subs. *berkhoffii*. However these amplicons were much shorter than expected for *B. vinsonii* subspecies *berkhoffii* genotype II and genotype IV sequences in GenBank. Because Pap31 gene amplification was unsuccessful for these samples, it is unclear whether small amplicons represent a species related to *B. vinsonii* subs. *berkhoffii* or a new *Bartonella* sp.

<table>
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<tr>
<th>Table. Primers used for PCR amplification</th>
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<tr>
<td>Primer</td>
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<tr>
<td>28s s</td>
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<td>28s as</td>
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<tr>
<td>ITS 325s</td>
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<td>ITS 1100as</td>
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<td>Papi 31s</td>
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<td>Papi 31 688s</td>
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