A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster


Summary

Background An ongoing outbreak of pneumonia associated with a novel coronavirus was reported in Wuhan city, Hubei province, China. Affected patients were geographically linked with a local wet market as a potential source. No data on person-to-person or nosocomial transmission have been published to date.

Methods In this study, we report the epidemiological, clinical, laboratory, radiological, and microbiological findings of five patients in a family cluster who presented with unexplained pneumonia after returning to Shenzhen, Guangdong province, China, after a visit to Wuhan, and an additional family member who did not travel to Wuhan. Phylogenetic analysis of genetic sequences from these patients were done.

Findings From Jan 10, 2020, we enrolled a family of six patients who travelled to Wuhan from Shenzhen between Dec 29, 2019 and Jan 4, 2020. Of six family members who travelled to Wuhan, five were identified as infected with the novel coronavirus. Additionally, one family member, who did not travel to Wuhan, became infected with the virus after several days of contact with four of the family members. None of the family members had contacts with Wuhan markets or animals, although two had visited a Wuhan hospital. Five family members (aged 36–66 years) presented with fever, upper or lower respiratory tract symptoms, or diarrhoea, or a combination of these 3–6 days after exposure. They presented to our hospital (The University of Hong Kong-Shenzhen Hospital, Shenzhen, Hubei province, China) 6–10 days after symptom onset. They and one asymptomatic child (aged 10 years) had radiological ground-glass lung opacities. Older patients (aged >60 years) had more systemic symptoms, extensive radiological ground-glass lung changes, lymphopenia, thrombocytopenia, and increased C-reactive protein and lactate dehydrogenase levels. The nasopharyngeal or throat swabs of these six patients were negative for known respiratory microbes by point-of-care multiplex RT-PCR, but five patients (four adults and the child) were RT-PCR positive for genes encoding the internal RNA-dependent RNA polymerase and surface Spike protein of this novel coronavirus, which were confirmed by Sanger sequencing. Phylogenetic analysis of these five patients’ RT-PCR amplicons and two full genomes by next-generation sequencing showed that this is a novel coronavirus, which is closest to the bat severe acute respiratory syndrome (SARS)-related coronaviruses found in Chinese horseshoe bats.

Interpretation Our findings are consistent with person-to-person transmission of this novel coronavirus in hospital and family settings, and the reports of infected travellers in other geographical regions.

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Introduction

The Health Commission of Hubei province, China, first announced a cluster of unexplained cases of pneumonia on Dec 31, 2019. 27 patients were initially reported, which was subsequently revised to 41 on Jan 11, 2020, with seven severe cases and one death. Some patients were reported to have radiographic ground-glass lung changes; normal or lower than average white blood cell lymphocyte, and platelet counts; hypoxaemia; and deranged liver and renal function. Most were said to be geographically linked to the Huanan seafood wholesale market, which was subsequently reported by journalists to be selling freshly slaughtered game animals. To date, no evidence of person-to-person transmission or affected health-care workers has been published in the scientific literature. The Chinese health authority said that the patients initially tested negatively for common respiratory viruses and bacteria, but later tested positive for a novel coronavirus. The virus was soon isolated and its genome sequenced by a number of Chinese

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Evidence before this study
We searched PubMed on Jan 13, 2020, with no starting date limitations, using the terms “family”, “pneumonia”, “Wuhan”, “coronavirus”, and “novel” for articles in English. Our search did not reveal any reports of novel coronavirus pneumonia in Wuhan before 2020. We only noted family clusters of pneumonia due to the novel severe acute respiratory syndrome (SARS) coronavirus in 2003, and Middle East respiratory syndrome coronavirus in 2012.

Added value of this study
The epidemiological, clinical, laboratory, radiological, and microbiological findings of unexplained pneumonia in a Shenzhen family cluster connected to a Wuhan hospital were presented. The diagnostic tests from relevant clinical samples confirmed the presence of a novel coronavirus in five of six patients with radiological changes of viral pneumonia. The phylogenetic analysis of this novel coronavirus suggested its linkage to a possible animal source.

Methods
Cases
On Jan 10, 2020, we initially enrolled two patients who initially presented to The University of Hong Kong-Shenzhen Hospital (Shenzhen, Guangdong province, China) with fever, respiratory symptoms, and pulmonary infiltrates on chest radiographs. Subsequently, between Jan 11, and Jan 15, 2020, five other members of this family also presented to our hospital for the assessment of their health conditions.

We recorded and analysed the history, physical findings, and haematological, biochemical, radiological, and microbiological investigation results. All laboratory procedures for clinical samples have been previously reported. Briefly, nasopharyngeal and throat swabs and stool and urine samples were taken and put into viral transport media. Plasma was separated from EDTA bottles and serum were separated from clotted blood bottles.

This study was approved by the Institutional Review Board of The University of Hong Kong-Shenzhen Hospital (number [2015]90). We obtained written consent from the patients.

Respiratory and diarrhoeal pathogen detection
Respiratory samples of the patients were tested for influenza A and B viruses and respiratory syncytial virus using the Xpert Xpress Flu/RSV assay (Cepheid, Sunnyvale, CA, USA) according to the manufacturer’s instructions. To detect the presence of 18 respiratory virus targets and four bacteria (including adenovirus, coronaviruses [HCoV-229E, HCoV-NL63, HCoV-Oc43, HCoV-HKU1, and MERS-CoV], human metapneumovirus, respiratory syncytial virus, human rhinovirus or enterovirus, influenza A viruses [H1, H1-2009 and H3], influenza B virus, parainfluenza viruses [types 1–4], Bordetella pertussis, Bordetella parapertussis, Chlamydophila pneumoniae, and Mycoplasma pneumoniae), samples were tested using BioFire FilmArray Respiratory Panel 2 plus (bioMérieux, Marcy l’Etoile, France) according to the manufacturer’s instructions. The two faecal samples were taken from the patients who had diarrhoea as part of their symptoms, and the samples were tested by BioFire FilmArray Gastrointestinal panel (bioMérieux) for 22 diarrhoeal pathogens.

Reverse transcription, in-house conventional RT-PCR and sequencing
Reverse transcription was done using the SuperScript IV reverse transcriptase (Invitrogen, Carlsbad, USA) as previously described. The reaction mixture (10 μL) contained 5–5 μL of RNA, 2 μL of 5× SuperScript IV buffer, 0–5 μL of 100 mM dithiothreitol, 0–5 μL of 10 mM deoxynucleotide triphosphate (dNTP) mixture, 0–5 μL of 50 μM random hexamers, 0–5 μL of SuperScript IV reverse transcriptase (200 U/μL), and 0–5 μL of RNase-free water. The mixtures were incubated at 23°C for 10 min, followed by 50°C for 10 min and 80°C for 10 min. The PCR mixture (25 μL) contained 1 μL of cDNA, 2–5 μL of 10× PCR buffer II, 2 μL of 25 mM MgCl₂, 0–5 μL of 10 mM dNTP mix, 2–5 μL of each 10 μM forward and reverse primer, 0–125 μL of AmpliTaq Gold Polymerase (Applied Biosystems, Foster City, USA; 5 U/μL), and nuclease-free water.

The first set of primers was the forward primer (5’-CAAGTTGGGTAAGGCTAGACCTT-3’) and the reverse primer (5’-ACTTAGGATAAATCCCAACCAT-3’) targeting 344 bp of RNA-dependent RNA polymerase (RdRp) gene of all severe acute respiratory syndrome (SARS)-related coronaviruses. The second set of primers was designed after our first 2019-nCoV genome sequence by Nanopore sequencing from
Figure 1: Chronology of symptom onset of the Shenzhen family cluster and their contacts in Wuhan. Dates filled in red are the dates on which patients 1–6 had close contacts with their relatives (relatives 1–5). Dates filled in yellow are the dates on which patients 3–6 stayed with patient 7. The boxes with an internal red cross are the dates on which patients 1 and 3 or relatives 1, 2, and 3 had stayed overnight (white boxes) at or had visited (blue boxes) the hospital in which relative 1 was admitted for febrile pneumonia. The information of relatives 1–5 was provided by patient 3. No virological data were available.
<table>
<thead>
<tr>
<th>Relationship</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
<th>Patient 5</th>
<th>Patient 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother of patient 3</td>
<td>Father of patient 3</td>
<td>Daughter of patients 1 and 2</td>
<td>Son-in-law of patients 1 and 2</td>
<td>Grandson of patients 1 and 2</td>
<td>Mother of patient 4 in Shenzhen</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>65</td>
<td>66</td>
<td>37</td>
<td>36</td>
<td>30</td>
<td>63</td>
</tr>
<tr>
<td>Sex</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Occupation</td>
<td>Retired</td>
<td>Retired</td>
<td>Office worker</td>
<td>Architect</td>
<td>Student</td>
<td>Retired</td>
</tr>
<tr>
<td>Chronic medical illness</td>
<td>Hypertension; benign intracranial tumour treated by gamma knife</td>
<td>Hypertension</td>
<td>None</td>
<td>Chronic sinusitis</td>
<td>None</td>
<td>Diabetes</td>
</tr>
<tr>
<td>Interval between symptom onset and arrival at Wuhan (days)</td>
<td>5 (hospital exposure)</td>
<td>6 (hospital exposure)</td>
<td>4 (hospital exposure)</td>
<td>3</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Interval between admission to hospital and symptom onset (days)</td>
<td>7</td>
<td>6</td>
<td>9</td>
<td>10</td>
<td>NA</td>
<td>7</td>
</tr>
<tr>
<td>Presenting symptoms and signs</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fever</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cough</td>
<td>+ (dry)</td>
<td>+ (dry)</td>
<td>-</td>
<td>+ (productive)</td>
<td>-</td>
<td>+ (dry)</td>
</tr>
<tr>
<td>Generalised weakness</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nasal congestion</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rhinorrhoea</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sore throat</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pleuritic chest pain</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>-</td>
<td>-</td>
<td>+ (3 days, 5-6 times per day)</td>
<td>+ (4 days, 7-8 times per day)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Body temperature (°C)</td>
<td>39·0</td>
<td>39·0</td>
<td>36·2</td>
<td>36·5</td>
<td>36·5</td>
<td>39·0</td>
</tr>
<tr>
<td>Oximetry saturation (%)</td>
<td>94%</td>
<td>96%</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>13·1</td>
<td>15·6</td>
<td>15·0</td>
<td>15·2</td>
<td>14·6</td>
<td>13·0</td>
</tr>
<tr>
<td>White blood cell count (×10⁹ cells per L)</td>
<td>(normal range 3·9-9·9)</td>
<td>4·8</td>
<td>4·2</td>
<td>5·6</td>
<td>11·4 (↑)</td>
<td>6·5</td>
</tr>
<tr>
<td>Neutrophil count (×10⁹ cells per L)</td>
<td>(normal range 2·0-7·4)</td>
<td>4·0</td>
<td>3·2</td>
<td>3·1</td>
<td>8·1 (↑)</td>
<td>3·2</td>
</tr>
<tr>
<td>Lymphocyte count (×10⁹ cells per L)</td>
<td>(normal range 1·1-3·6)</td>
<td>0·6 (↑)</td>
<td>0·7 (↑)</td>
<td>2·2</td>
<td>2·7</td>
<td>2·8</td>
</tr>
<tr>
<td>Platelet count (×10⁹ cells per L)</td>
<td>(normal range 152-341)</td>
<td>157 (↑)</td>
<td>118 (↑)</td>
<td>224</td>
<td>196</td>
<td>197</td>
</tr>
<tr>
<td>Prothrombin time (s)</td>
<td>(normal range 11·0-14·5)</td>
<td>12·6</td>
<td>12·5</td>
<td>13·0</td>
<td>13·0</td>
<td>13·1</td>
</tr>
<tr>
<td>International normalised ratio</td>
<td>1·0</td>
<td>1·0</td>
<td>1·0</td>
<td>1·0</td>
<td>1·0</td>
<td>1·0</td>
</tr>
<tr>
<td>Activated partial thromboplastin time (s)</td>
<td>(normal range 26·0-40·0)</td>
<td>45·4 (↑)</td>
<td>45·3 (↑)</td>
<td>36·0</td>
<td>31·4</td>
<td>34·0</td>
</tr>
<tr>
<td>D-dimer (µg/mL)</td>
<td>0·6 (↑)</td>
<td>0·3</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0·6 (↑)</td>
</tr>
<tr>
<td>Fibrinogen (g/dL)</td>
<td>6·2 (↑)</td>
<td>5·1 (↑)</td>
<td>3·8</td>
<td>3·8</td>
<td>2·9</td>
<td>4·5 (↑)</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>55·6 (↑)</td>
<td>34·2 (↑)</td>
<td>0·5</td>
<td>4·9</td>
<td>0·2</td>
<td>44·9 (↑)</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>39·4</td>
<td>38·5</td>
<td>50·4</td>
<td>48·1</td>
<td>49·1</td>
<td>41·2</td>
</tr>
<tr>
<td>Bilirubin (µmol/L)</td>
<td>6·9</td>
<td>5·9</td>
<td>9·3</td>
<td>8·9</td>
<td>3·6</td>
<td>10·4</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/L)</td>
<td>68</td>
<td>56</td>
<td>56</td>
<td>48</td>
<td>211 (↑)</td>
<td>66</td>
</tr>
<tr>
<td>Alanine aminotransferase (U/L)</td>
<td>(normal range 35·0-105)</td>
<td>29·5</td>
<td>23·3</td>
<td>27·4</td>
<td>18·1</td>
<td>28·2</td>
</tr>
<tr>
<td>Aspartate aminotransferase (U/L)</td>
<td>(normal range 0·0-32·0)</td>
<td>20·5</td>
<td>23·3</td>
<td>27·4</td>
<td>18·1</td>
<td>28·2</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>(normal range 2·8-8·1)</td>
<td>3·5</td>
<td>5·7</td>
<td>3·1</td>
<td>5·2</td>
<td>5·6</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>(normal range 44·4-80)</td>
<td>5·3</td>
<td>93 (↑)</td>
<td>67</td>
<td>87 (↑)</td>
<td>51</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>(normal range 136-145)</td>
<td>136</td>
<td>133 (↑)</td>
<td>142</td>
<td>141</td>
<td>141</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>(normal range 3·5-5·1)</td>
<td>3·2 (↑)</td>
<td>3·7</td>
<td>3·7</td>
<td>3·7</td>
<td>3·9</td>
</tr>
<tr>
<td>Creatine kinase (U/L)</td>
<td>(normal range 0-1·70)</td>
<td>42</td>
<td>109</td>
<td>50</td>
<td>127</td>
<td>78</td>
</tr>
<tr>
<td>Lactate dehydrogenase (U/L)</td>
<td>(normal range 125-214)</td>
<td>286 (↑)</td>
<td>232 (↑)</td>
<td>192</td>
<td>176</td>
<td>194</td>
</tr>
<tr>
<td>Amylase (U/L)</td>
<td>(normal range 26-100)</td>
<td>NA</td>
<td>NA</td>
<td>70</td>
<td>61</td>
<td>61</td>
</tr>
</tbody>
</table>

NA=not available. + = positive. - = negative. ↑ = above normal range. ↓ = below normal range.

Table 1: Summary of clinical features and laboratory results of the family cluster infected with 2019 novel coronavirus, at presentation.
In-house one-step real-time RT-PCR assay
A total of 140 µL of respiratory, urine, stool, serum, or plasma samples from each patient was subjected to RNA extraction into 50 µL elutes using QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany). Forward (5′-CTACTAATTAAAAATGATCTCTCTTATTACT-3′) and reverse (5′-CAAGCTATAACGCCAGCTGA-3′) primers targeting the S gene of this novel coronavirus were used for the assay. Real-time RT-PCR assay was done using Quantitative SYBR Green RT-PCR Kit (Qiagen) in a LightCycler 480 Real-Time PCR System (Roche, Basel, Switzerland), as previously described. Each 20 µL reaction mixture contained 10 µL of 2×QuantiNova SYBR Green RT-PCR Master Mix, 0.2 µL of QN SYBR Green RT-Mix, 1 µM of each 10 µM forward and reverse primers, and 5 µL of RNA and nuclease-free water. Reactions were incubated at 50°C for 10 min and 95°C for 2 min, followed by 45 cycles at 95°C for 5 s and 60°C for 30 s, and then subjected to melting curve analysis (95°C for 5 s, 65°C for 1 min, followed by a gradual increase in temperature to 97°C with continuous recording of fluorescence).

Whole-genome sequencing and genome analysis by bioinformatics
Whole-genome sequencing was done using Oxford Nanopore MinION device (Oxford Nanopore Technologies, Oxford, UK) supplemented by Sanger sequencing. RNA was extracted from host cell-depleted nasopharyngeal and sputum samples using a QIAamp Viral RNA Mini Kit, as described previously. Whole-genome amplification of the coronavirus was done using a sequence-independent single-primer amplification approach, as described previously. Bioinformatics analyses were done using an in-house pipeline. Details on the library preparation and bioinformatics analysis are described in the appendix.
on Dec 12, 2019) according to the Chinese health authority. They had no history of contact with animals, visits to markets including the Huanan seafood wholesale market in Wuhan, or eating game meat in restaurants. The family stayed in the same hotel throughout their travel. Patients 1 and 2 stayed in one room and patients 3–6 stayed in another room. After patient 4 developed fever and diarrhoea on Jan 1, 2020, patients 5 and 6 stayed in the same room as patients 1 and 2, and patient 3 stayed with patient 4. Patients 1–6 had met with their relatives (relatives 2–5: one female cousin and three aunts of patient 3) every day during their stay in Wuhan for meals. Relative 4 made frequent visits to the wet market but not the Huanan seafood wholesale market, which had been implicated by the health authority to be the epicentre.

### Microbiological findings from clinical specimens collected from the family cluster infected with 2019 novel coronavirus, at presentation

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
<th>Patient 5</th>
<th>Patient 6</th>
<th>Patient 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional RT-PCR</td>
<td>7</td>
<td>6</td>
<td>9</td>
<td>10</td>
<td>NA</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Nasopharyngeal swab</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RdRp</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spike</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Throat swab</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stool</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Real-time RT-PCR (spike gene)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nasopharyngeal swab</td>
<td>+ (Ct 31)</td>
<td>+ (Ct 27)</td>
<td>+ (Ct 31)</td>
<td>+ (Ct 31)</td>
<td>+ (Ct 27)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Throat swab</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>ND</td>
<td>+ (Ct 40)</td>
<td>NA</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>NA</td>
<td>NA</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
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<tr>
<td>Stool</td>
<td>NA</td>
<td>NA</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FilmArray RP2 plus (nasopharyngeal swab only)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Xpert Xpress Flu/RSV (nasopharyngeal swab only)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>FilmArray GI panel (faecal sample only)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

**Table 2:** Microbiological findings from clinical specimens collected from the family cluster infected with 2019 novel coronavirus, at presentation

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Discussion

We report here a familial cluster of unexplained pneumonia due to 2019-nCoV. Six of seven family members had radiological changes of viral pneumonia, among whom five (patients 1, 2, 4, 5, and 7) tested positive for 2019-nCoV by RT-PCR. Five patients (patients 1, 2, 3, 4,
Shenzhen. For the patients’ relatives (relatives 2–5), they could have acquired the infection from the hospital or the community, although no virological confirmation was possible and they had no animal contacts, game food, or visits to the Huanan seafood wholesale market. Notably, patient 1 or patient 3 who had visited Wuhan hospital might have been infected before symptom onset because patient 5 was shedding virus without symptoms. These findings suggested that person-to-person transmission and intercity spread of 2019-nCoV by air travel are possible, supporting reports of infected Chinese travellers from Wuhan being detected in other geographical regions.

Many of the epidemiological, clinical, laboratory, and radiological features of this novel coronavirus pneumonia were similar to those of SARS patients in 2003,3,15,16. The incubation period of the Wuhan pneumonia appeared similar to that of SARS. The attack rate is rather high, up to 83% if we included the five patients (patients 1, 2, 3, 4, and 5) with unexplained ground-glass radiological changes of the lungs on CT scan as the case definition in this family outbreak after visiting Wuhan. A rather unexpected finding from the lung CT scan of patient 5, which was done on the insistence by the nervous parents, also showed ground-glass pneumonic changes. Patient 5 was later confirmed virologically to have an asymptomatic infection. Although asymptomatic patients with SARS were uncommon, they were documented in our retrospective study in the minor 2004 SARS outbreak after reopening of the wildlife market in Guangzhou.17 Notably, patients 3 and 4 were afebrile at presentation to our hospital. These cryptic cases of walking pneumonia might serve as a possible source to propagate the outbreak. Further studies on the epidemiological significance of these asymptomatic cases are warranted.

The symptoms of this novel pneumonia were also nonspecific. The three oldest patients in this family with comorbidities had more severe systemic symptoms of generalised weakness and dry cough. As expected, they might have decreased total white blood cell, lymphocyte, or platelet counts, with also extended activated thromboplastin time and increased C-reactive protein level. The multifocal ground-glass changes on lung CT scan were typical of viral pneumonia. Their lung involvement was also more diffuse and extensive than those of the younger patients, whose blood test results were largely normal. Patient 4, who had a history of chronic sinusitis, might have a bacterial superinfection because he had a productive cough instead of a dry cough. He also had a high white blood cell count, although the bacterial test was negative.

Interestingly, the two younger adults (patients 3 and 4) initially had diarrhoea, which was also reported in 10–6% (15 of 142) of our SARS patients at presentation;18 however, the subsequent faecal samples of patients 3 and 4 that were collected 9–10 days after symptom onset were negative for the virus after the diarrhoea had long subsided. Up to 30% of patients with Middle East respiratory syndrome coronavirus (MERS-CoV) also have
Subgenomic RNA indicating viral replication was seen in faecal samples of patients with MERS. Moreover, MERS-CoV was shown to survive in simulated fed gastrointestinal juice and the ability to infect intestinal organoid models. Diarrhoea and gastrointestinal involvement are well known in coronavirus infections of animals and humans.

On microbiological testing, we did not find any evidence of other known respiratory viral or bacterial infections, but specific RT-PCR assays for two widely separated genome targets—the highly conserved RdRp and the highly variable S genes—were positive for this novel 2019-nCoV. Two complete genome sequences of this novel coronavirus were recovered from the nasopharyngeal swab of patient 2 and the sputum sample of patient 5 with an earlier cycle threshold value indicating a higher viral load. Patient 2 had undergone more comorbidities and clinical features and radiological findings of more severe disease than the other patients included here. Moreover, the serum sample of patient 2 was also positive for 2019-nCoV, which might indicate some virus spillover from the more severely infected lung into the systemic circulation, as previously reported in patients with SARS. Sputum samples were available for testing from patients 5 and 7. The cycle threshold values of the sputum samples were 8–13 cycles earlier than those of throat swabs, indicating higher viral loads detected in the lower respiratory tract. This finding is consistent with the observations in patients with MERS who had higher viral loads in lower respiratory tract samples than in upper respiratory tract samples. Thus, repeat testing of upper respiratory tract samples or testing of lower respiratory tract samples are warranted in clinically suspected cases with an initially negative result in nasopharyngeal or throat swab. Unlike our patients in the 2003 SARS outbreak, we found no evidence of viral shedding in urine and faeces in these six patients. However, improved systematic serial collection and testing of an increased number of such samples is warranted.

Coronaviruses are enveloped, positive-sense, single-stranded RNA viruses, capable of rapid mutation and recombination. They are classified into alphacoronaviruses and betacoronaviruses, which both have their gene source from bats and are mainly found in mammals such as bats, rodents, civets, and humans; and gammacoronaviruses and deltacoronaviruses, which both have their gene source from birds and are mainly found in birds. Phylogenetic analysis of the PCR amplicon fragments from five of our six patients and the complete virus genome of 29·8 kilobases from patients 2 and 5 showed that the virus is a novel betacoronavirus belonging to the lineage B or subgenus sarbecovirus, which also includes the human SARS coronavirus. The genome of our virus strains are phylogenetically closest to the bat SARS-related coronaviruses first found in the Chinese horseshoe bats, *Rhinolophus sinicus*, captured in Zhusuan, Zhejiang province, China, between 2015 and 2017. Notably, the first SARS-related coronavirus was also discovered in the *R sinicus* found in Hong Kong, and central and south China in 2005. The full virus genome had about an 89% nucleotide identity with bat-SL-CoVZC45, which makes it a new species. Moreover, the Spike protein of our virus has an 84% nucleotide identity with the bat-SL-CoVZC45 coronavirus and an 78% nucleotide identity with the human SARS coronavirus. Although substantial genetic differences exist between this and other betacoronaviruses, cross reactions in RT-PCR or antibody assays for SARS or other betacoronaviruses are possible if the primers and antigens are not carefully chosen, as previously reported. Further studies on the optimal diagnostic tests are warranted.

In summary, an outbreak of novel coronavirus is ongoing at Wuhan in the winter of 2019–20. Similar to the 2003 SARS outbreak in Guangzhou, Wuhan is also a rapidly flourishing capital city of the Hubei province and the traffic hub of central China. Moreover, both outbreaks were initially connected to wet markets where game animals and meat were sold. In the case of SARS, person-to-person transmission was efficient and super-spreading events had led to major outbreaks in hotels and hospitals. Learning from the SARS outbreak, which started as animal-to-human transmission during the first phase of the epidemic, all game meat trades should be optimally regulated to terminate this portal of transmission. But as
shown in this study, it is still crucial to isolate patients and trace and quarantine contacts as early as possible because asymptomatic infection appears possible (as shown in one of our patients), educate the public on both food and personal hygiene, and alert health-care workers on compliance to infection control to prevent superspreading events. Unlike the 2003 SARS outbreak, the improved surveillance network and laboratory capability of China was able to recognise this outbreak within a few weeks and announced the virus genome sequences that would allow the development of rapid diagnostic tests and efficient epidemiological control. Our study showed that person-to-person transmission in family homes or hospital, and intercity spread of this novel coronavirus are possible, and therefore vigilant control measures are warranted at this early stage of the epidemic.

Contributors
JF-WC and K-YY had roles in the study design, clinical management, patient recruitment, data collection, data analysis, data interpretation, literature search, and writing of the manuscript. SY, K-HK, KK-WT, HCwu, CC-YW, RW-BP, H-WT, SK-FL, K-HC, YK-MP, WMC, JDI, J-PC, VC-CC, and HChe had roles in the experiments, data collection, data analysis, and data interpretation. JY, CK-MH, FX, and JL had roles in recruitment, data collection, and clinical management. All authors reviewed and approved the final version of the manuscript.

Declaration of interests
We declare no competing interests.

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