Foodborne Outbreaks Caused by Human Norovirus GII.P17-GII.17–Contaminated Nori, Japan, 2017

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Seven foodborne norovirus outbreaks attributable to the GII.P17-GII.17 strain were reported across Japan in 2017, causing illness in a total of 2,094 persons. Nori (dried shredded seaweed) was implicated in all outbreaks and tested positive for norovirus. Our data highlight the stability of norovirus in dehydrated food products.

Seven foodborne norovirus outbreaks were reported in 4 remote areas across Japan during January–February 2017, causing illness in 2,094 persons (Figure). In all outbreaks, norovirus GII.P17-GII.17 was detected in stool specimens of patients. Food survey results indicated that dried shredded seaweed (nori) was served before all outbreaks. Norovirus was also detected in nori-containing dishes. An investigation revealed that the nori served in each instance was manufactured by the same food processing company.

The Study

Outbreak 1 was reported on January 26 in 4 kindergartens, 6 elementary schools, and 5 junior high schools in the prefecture of Wakayama, Japan (1). A total of 1,943 children and 119 school staff members had eaten lunch the day before; of these, 678 (34.9%) children and 85 (71.4%) school staff members had acute gastroenteritis. Norovirus was also detected in the stool specimens from 10 of the 27 food handlers in the central kitchen who had eaten the same lunch. Food survey results indicated that boiled vegetables mixed with shredded nori was responsible for this outbreak.

Outbreak 2 was reported on February 17 in 7 elementary schools in the city of Tachikawa, part of metropolitan Tokyo, Japan (1). A total of 1,084 (35.2%) of the 3,078 persons who ate the lunch served the day before had gastrointestinal symptoms. The lunch included shredded nori as a topping on cooked rice.

Outbreaks 3 and 4 were reported in separate, self-catered school lunch settings on February 22 and 25 in the city of Kodaira, also part of metropolitan Tokyo (1). The numbers of patients with gastrointestinal symptoms were 26 (5.6% attack rate) in outbreak 3 and 81 (12.6% attack rate) in outbreak 4. Shredded nori was served as a topping on cooked rice in both outbreaks.

Outbreak 5 was reported in Western Tama, also in metropolitan Tokyo, on February 27. A central kitchen served school lunch to 19 persons, and 2 of them (10.5%) had gastrointestinal symptoms. Shredded nori was served with boiled vegetables.

Outbreak 6 was reported on March 9. After conducting retrospective surveillance, the Kurume city government in Fukuoka prefecture announced that a foodborne outbreak occurred in a business office on January 25 that was attributable to a nori product with the same expiration date as the nori implicated in outbreaks 1–5. A total of 39 (92%) of the 42 employees who had eaten at the office’s café had gastrointestinal symptoms. Shredded nori had been served as a salad topping.

Outbreak 7, announced by the Osaka prefectural government, caused illness in 99 persons during February 18–24, including 4 food handlers who consumed a bento box (a single-portion take-out or home-packed meal common in Japanese cuisine). A bento shop provided 228 meal boxes during this period that contained nori product from company B. Those who had eaten from bento boxes without nori also had gastrointestinal symptoms.

We detected norovirus in nori-containing food in outbreak 1 by using the PANSORBIN-trap method (2).
Tokyo Metropolitan Institute of Public Health tested norovirus from food from outbreaks 2–5 by using the A3T method, by which nori was incubated with the Proteus vulgaris NBRC3045 strain. Among 21 nori samples, including those from the merchandise in stock of the wholesaler and those that remained in the kitchen from outbreaks 2–5 in Tokyo, 7 samples were positive for norovirus GII.17 by nested reverse transcription PCR. These data, along with the food survey results, strongly suggest that nori was responsible for the food-borne norovirus outbreaks.

The amount of shredded nori served as a topping on cooked rice was 0.5–1.0 g per dish. The shredded nori contained 360–2,900 copies/g of norovirus genome, a measure that did not take into account the recovery rate of the virus particles from the food. In outbreak 7, food handlers shared and reused plastic gloves, which increased the risk for norovirus contamination across food.

Nori sheets were originally produced by company C, and food processing company B shredded them to 2-mm width and packed them with desiccants. Approximately 800 packages were produced, shipped to company A on December 10 and 27, 2016, and sold under the company A brand with the same expiration date. Another batch was produced by company B in December 2016 and sold under its own brand with an expiration date of May 31, 2017. These packages were stored at an ambient temperature.

On February 27, 2017, the Osaka city government announced that norovirus GII.17 was detected from 8 of 25 environmental wipe samples at company B. These samples were subjected to ultracentrifugation to precipitate norovirus particles before nucleic acid extraction. Samples collected from the nori sheet shredding equipment, a telephone handle, and the toilet were positive for norovirus. At company B, an employee who was responsible for the

Figure. Overview of foodborne norovirus outbreaks associated with dried shredded nori during the 2016–17 endemic season, Japan. Production and distribution of dried, shredded nori products and the 7 norovirus outbreaks are outlined. Solid and dashed lines indicate the production and distribution of 2 distinct lots. The asterisk indicates detection of norovirus in food, shredded nori, or both. Inset map shows geographic locations of outbreaks and manufacturers.
recovery of norovirus genomic RNA from food is not breaks in 4 remote regions of Japan were attributable to a
Our study demonstrated that 7 foodborne norovirus out
all 7 outbreaks were attributable to GII.P17-GII.17–
contaminated shredded nori.

These results and epidemiologic link indicated that
outbreaks formed a distinct cluster from the outbreaks un
were managed by each institute of public health. Y.M. and H.S.
designed specific primers for GII.P17-GII.17. K.N., H.K., and
were shown to be useful for this purpose. Sequence analy
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norovirus have been reported previously. In 2014, a large
norovirus outbreak involving 1,271 persons was reported
in which bread was contaminated with norovirus. The con
aminated bread was served at school lunch within 2 days
after production (9). In contrast, in these nori-related out
breaks, the traceback of implicated nori product revealed
that the norovirus infectivity remained for ≥2 months at
ambient temperature under dry conditions. However, the
percentage of persons with gastrointestinal symptoms
gradually decreased from the date of nori production, sug
suggesting a decline in norovirus infectivity over time under
dry conditions.

Acknowledgments
We are grateful to Mamoru Noda for information on the
PANSORBIN-trap and A3T methods

Conclusions
Our study demonstrated that 7 foodborne norovirus out
breaks in 4 remote regions of Japan were attributable to a
shredded nori product that was most likely contaminated
during manufacturing. From a technical standpoint, the
recovery of norovirus genomic RNA from food is not
efficient, so identifying the contaminated food can be dif
however, the PANSORBIN-trap and A3T methods
were shown to be useful for this purpose. Sequence analy
sis of the RdRp and VP1 regions of the norovirus genome
enabled us to distinguish nori-related outbreaks involving
norovirus GII.P17-GII.17 from other outbreaks involving
the same genotype.

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Author contributions: Detection of norovirus and genetic analysis
were managed by each institute of public health. Y.M. and H.S.
designed specific primers for GII.P17-GII.17. K.N., H.K., and
N.S. conducted the phylogenetic tree analysis. N.S. and J.K.
wrote the manuscript. All authors commented on the manuscript.

About the Author
Dr. Sakon is a senior research scientist whose primary research
interests include public health and viral infections of the
gastrointestinal tract.

Table. Genetic analysis of norovirus GII.P17-GII.17 isolates detected in clinical specimens from outbreaks during the 2016–17 endemic season, Japan*

<table>
<thead>
<tr>
<th>Outbreak or strain</th>
<th>GenBank accession nos.</th>
<th>Isolation date</th>
<th>Identity with outbreak 1 strain, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outbreak 1</td>
<td>LC318755–LC318758</td>
<td>Jan 2017</td>
<td>NA</td>
</tr>
<tr>
<td>Outbreak 2</td>
<td>LC258403†</td>
<td>Feb 2017</td>
<td>100</td>
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<tr>
<td>Outbreak 6</td>
<td>LC311767–LC311773</td>
<td>Jan 2017</td>
<td>100</td>
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<tr>
<td>Outbreak 7</td>
<td>LC318751–LC318754</td>
<td>Feb 2017</td>
<td>100</td>
</tr>
<tr>
<td>Outbreak A:16–064</td>
<td>LC318745</td>
<td>May 2016</td>
<td>99.6</td>
</tr>
<tr>
<td>Outbreak B:16–299</td>
<td>LC318746</td>
<td>Nov 2016</td>
<td>99.9</td>
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<td>Outbreak C:16–292</td>
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<td>Outbreak D:16–315</td>
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<tr>
<td>GII.17 Kawasaki308</td>
<td>LC037415</td>
<td>Feb 2015</td>
<td>99.6</td>
</tr>
</tbody>
</table>

*NA, not applicable; ND, not done.
†This isolate was registered as a representative strain of outbreaks 2–5 because all the isolates from outbreaks 2–5 showed 100% sequence identity.
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Technical Appendix

Technical Appendix Figure. Phylogenetic tree analysis of norovirus GII.P17-GII.17 strains associated with dried shredded nori–related and unrelated outbreaks during the 2016–17 endemic season, Japan, 2017. Analyses of viral genes encoding RdRp (A) and VP1 (B) are shown. Solid circles indicate strains from outbreaks with shredded nori. GII.P17 or GII.17_Wakayama/2017 is represented by LC318755, 1 of the 4 strains detected in outbreak 1 (LC318755–LC318758). GII.P17 or GII.17_Tokyo/2017 is represented by LC258403, 1 of the strains detected during outbreaks 2–5. GII.P17 or GII.17_Fukuoka/2017 is represented by LC311767, and GII.P17 or GII.17_Osaka/2017 is represented by LC318751 (Table). The evolutionary analysis was conducted by using MEGA7 software, with the maximum-likelihood method based on the Tamura-Nei model. The phylogenetic tree is drawn to scale, with branch lengths reflecting the number of substitutions per site.