

Supplemental Materials

Supplementary Table 1. Clinical and demographic characteristics of cases associated with the norovirus GI.5[P4] foodborne outbreak, Murcia, Spain, 2021. First part of outbreak includes from 21 July to 9 August and second part from 18-22 August.

| Characteristics | Value | |
|-----------------------------|----------------------------|-----------------------------|
| Onset of illness | July 21 | |
| Total no. symptomatic cases | 163 | |
| Hospitalized | 0 | |
| Died | 0 | |
| Duration of illness, days | 2 | |
| Sex | No. cases (%) | |
| M | 74 (42.8) | |
| F | 99 (57.2) | |
| Age range (years) | Value | |
| | 2-74 | |
| Age group (years) | No. cases | |
| | First part of the outbreak | Second part of the outbreak |
| 0-14 | 8 | 0 |
| 15-64 | 80 | 23 |
| >65 | 3 | 2 |
| Unknown | 25 | 32 |
| Symptom | % cases | |

| | First part of the outbreak | Second part of the outbreak |
|----------------|-----------------------------------|------------------------------------|
| Diarrhea | 79.2 | 84.0 |
| Nausea | 87.3 | 64.0 |
| Headache | 43.4 | 0 |
| Abdominal pain | 74.7 | 84.0 |
| Fever | 37.7 | 4.0 |
| Vomiting | 81.2 | 72.0 |
| Chills | 26.9 | 0 |

Supplementary table 2. Analysis of implicated food and drink in a Norovirus GI outbreak, in a hotel in Spain.

| Food and drink items | Exposed (Ate food or drink) | | | Unexposed (Did not eat food or drink) | | | Relative Risk | (95% CI) | p value |
|---------------------------|-----------------------------|-------|-------------|---------------------------------------|-------|-------------|---------------|------------------|---------|
| | Ill | Total | Attack rate | Ill | Total | Attack rate | | | |
| Drinks_Beach Bar | 14 | 21 | 66,7 | 6 | 12 | 50,0 | 1,33 | 0.7020 to 2.5326 | 0.3795 |
| Ice | 12 | 20 | 60,0 | 8 | 13 | 61,5 | 0,98 | 0.5574 to 1.7056 | 0.9293 |
| Beer | 6 | 11 | 54,5 | 14 | 22 | 63,6 | 0,86 | 0.4587 to 1.6016 | 0.6289 |
| Mineral Water Dispenser | 12 | 18 | 66,7 | 8 | 15 | 53,3 | 1,25 | 0.7033 to 2.2218 | 0.4470 |
| Coffee | 10 | 17 | 58,8 | 10 | 16 | 62,5 | 0,94 | 0.5431 to 1.6309 | 0.8289 |
| Soft Drink Dispenser | 9 | 14 | 64,3 | 11 | 18 | 61,1 | 1,05 | 0.6149 to 1.7996 | 0.8533 |
| Sausage | 1 | 4 | 25,0 | 19 | 28 | 67,9 | 0,37 | 0.0662 to 2.0502 | 0.2542 |
| Roasted Chicken | 6 | 9 | 66,7 | 14 | 23 | 60,9 | 1,10 | 0.6216 to 1.9297 | 0.7529 |
| Bacon | 6 | 9 | 66,7 | 14 | 23 | 60,9 | 1,10 | 0.6216 to 1.9297 | 0.7529 |
| Beef With Sauce | 8 | 10 | 80,0 | 12 | 22 | 54,5 | 1,47 | 0.8972 to 2.3976 | 0.1267 |
| Boiled Jam | 9 | 15 | 60,0 | 11 | 18 | 61,1 | 0,98 | 0.5644 to 1.7080 | 0.9482 |
| Grilled Beef | 5 | 9 | 55,6 | 15 | 23 | 65,2 | 0,85 | 0.4420 to 1.6419 | 0.6320 |
| Salad Served Just Made | 13 | 20 | 65,0 | 7 | 12 | 58,3 | 1,11 | 0.6262 to 1.9828 | 0.7128 |
| Pickle | 5 | 7 | 71,4 | 15 | 25 | 60,0 | 1,19 | 0.6750 to 2.0997 | 0.5470 |
| Olives | 8 | 11 | 72,7 | 12 | 21 | 57,1 | 1,27 | 0.7583 to 2.1362 | 0.3614 |
| Lettuce | 12 | 18 | 66,7 | 8 | 14 | 57,1 | 1,17 | 0.6671 to 2.0405 | 0.5889 |
| Onion | 7 | 10 | 70,0 | 12 | 21 | 57,1 | 1,23 | 0.7072 to 2.1220 | 0.4691 |
| Sweet Corn | 8 | 14 | 57,1 | 12 | 18 | 66,7 | 0,86 | 0.4901 to 1.4991 | 0.5889 |
| Tomato | 14 | 21 | 66,7 | 6 | 11 | 54,5 | 1,22 | 0.6585 to 2.2685 | 0.5248 |
| Cucumber | 8 | 13 | 61,5 | 12 | 19 | 63,2 | 0,97 | 0.5621 to 1.6890 | 0.9263 |
| Asparagus | 5 | 8 | 62,5 | 15 | 24 | 62,5 | 1,00 | 0.5380 to 1.8586 | 1 |
| Melon | 15 | 23 | 65,2 | 5 | 9 | 55,6 | 1,17 | 0.6091 to 2.2626 | 0.6320 |
| Watermelon | 13 | 20 | 65,0 | 7 | 12 | 58,3 | 1,11 | 0.6262 to 1.9828 | 0.7128 |
| Cheese | 14 | 19 | 73,7 | 6 | 14 | 42,9 | 1,72 | 0.8870 to 3.3327 | 0.1085 |
| Milk | 10 | 14 | 71,4 | 10 | 17 | 58,8 | 1,21 | 0.7236 to 2.0376 | 0.4622 |
| Fried Egg | 6 | 6 | 100,0 | 14 | 26 | 53,8 | 1,86 | 1.3010 to 2.6509 | 0.0007* |
| Toasted Bread | 14 | 18 | 77,8 | 6 | 13 | 46,2 | 1,69 | 0.8913 to 3.1863 | 0.1083 |
| Risotto With Mushrooms | 2 | 2 | 100,0 | 18 | 30 | 60,0 | 1,67 | 1.2444 to 2.2322 | 0.0006* |
| Carbonara Pasta | 5 | 7 | 71,4 | 15 | 25 | 60,0 | 1,19 | 0.6750 to 2.0997 | 0.5470 |
| Pizza | 13 | 20 | 65,0 | 7 | 12 | 58,3 | 1,11 | 0.6262 to 1.9828 | 0.7128 |
| Grilled Cheese And Tomato | 6 | 7 | 85,7 | 14 | 25 | 56,0 | 1,53 | 0.9656 to 2.4262 | 0.0701 |
| Seafood Paella | 10 | 19 | 52,6 | 10 | 14 | 71,4 | 0,74 | 0.4293 to 1.2646 | 0.2678 |
| Fish Paella | 5 | 9 | 55,6 | 11 | 19 | 57,9 | 0,96 | 0.4770 to 1.9303 | 0.9079 |
| Mussels | 1 | 2 | 50,0 | 19 | 30 | 63,3 | 0,79 | 0.1923 to 3.2415 | 0.7429 |
| Prawns | 2 | 7 | 28,6 | 18 | 25 | 72,0 | 0,40 | 0.1199 to 1.3130 | 0.1300 |
| Squid | 3 | 9 | 33,3 | 17 | 23 | 73,9 | 0,45 | 0.1735 to 1.1723 | 0.1023 |
| Shrimps | 2 | 4 | 50,0 | 18 | 28 | 64,3 | 0,78 | 0.2810 to 2.1529 | 0.6285 |
| Grilled Hake | 4 | 9 | 44,4 | 16 | 23 | 69,6 | 0,64 | 0.4075 to 2.0620 | 0.8334 |

| | | | | | | | | | |
|-------------------------------|----|----|-------|----|----|------|------|------------------|---------|
| Cheese Cake | 7 | 9 | 77,8 | 13 | 21 | 61,9 | 1,26 | 0.7741 to 2.0392 | 0.3556 |
| Icecream | 12 | 18 | 66,7 | 8 | 15 | 53,3 | 1,25 | 0.7033 to 2.2218 | 0.4470 |
| Rice With Milk (Dessert) | 5 | 7 | 71,4 | 15 | 25 | 60,0 | 1,19 | 0.6750 to 2.0997 | 0.5470 |
| Applee Pie | 7 | 10 | 70,0 | 13 | 22 | 59,1 | 1,18 | 0.6942 to 2.0214 | 0.5343 |
| Croissant | 11 | 15 | 73,3 | 9 | 17 | 52,9 | 1,39 | 0.8054 to 2.3823 | 0.2389 |
| Cutard | 7 | 10 | 70,0 | 13 | 22 | 59,1 | 1,18 | 0.6942 to 2.0214 | 0.5343 |
| Mayonnaise | 7 | 8 | 87,5 | 12 | 23 | 52,2 | 1,68 | 1.0473 to 2.6856 | 0.0314* |
| Oil | 15 | 22 | 68,2 | 5 | 10 | 50,0 | 1,36 | 0.6892 to 2.6981 | 0.3730 |
| Aubergine | 4 | 5 | 80,0 | 16 | 27 | 59,3 | 1,35 | 0.7880 to 2.3130 | 0.2746 |
| Broccoli | 6 | 11 | 54,5 | 14 | 21 | 66,7 | 0,82 | 0.4408 to 1.5186 | 0.5248 |
| Chips | 15 | 23 | 65,2 | 5 | 9 | 55,6 | 1,17 | 0.6091 to 2.2626 | 0.6320 |
| Boiled Egg | 2 | 3 | 66,7 | 18 | 28 | 64,3 | 1,04 | 0.4448 to 2.4177 | 0.9329 |
| Fritter | 10 | 17 | 58,8 | 10 | 14 | 71,4 | 0,82 | 0.4908 to 1.3819 | 0.4622 |
| Juice Dipenser | 12 | 17 | 70,6 | 8 | 14 | 57,1 | 1,24 | 0.7144 to 2.1361 | 0.4495 |
| Ice Drink | 9 | 9 | 100,0 | 11 | 22 | 50,0 | 2,00 | 1.3169 to 3.0375 | 0.0011* |
| Bolognese Pasta | 7 | 9 | 77,8 | 13 | 22 | 59,1 | 1,32 | 0.8041 to 2.1545 | 0.2744 |
| Surimi | 2 | 2 | 100,0 | 18 | 28 | 64,3 | 1,56 | 1.1803 to 2.0502 | 0.0017* |
| Burger | 4 | 6 | 66,7 | 15 | 23 | 65,2 | 1,02 | 0.5392 to 1.9380 | 0.9463 |
| Meat Paella | 10 | 17 | 58,8 | 9 | 14 | 64,3 | 0,92 | 0.5241 to 1.5977 | 0.7548 |
| Veal Stew | 4 | 5 | 80,0 | 12 | 13 | 92,3 | 0,87 | 0.5441 to 1.3805 | 0.5468 |
| Lamb | 6 | 6 | 100,0 | 8 | 10 | 80,0 | 1,25 | 0.9169 to 1.7041 | 0.1582 |
| Cream Cheese | 1 | 1 | 100,0 | 9 | 13 | 69,2 | 1,44 | 1.0053 to 2.0753 | 0.0467* |
| Hake With Green Sauce | 0 | 1 | 0,0 | 10 | 13 | 76,9 | 0,00 | 0.0297 to 3.7467 | 0.3735 |
| Swordfish | 0 | 1 | 0,0 | 10 | 13 | 76,9 | 0,00 | 0.0297 to 3.7467 | 0.3735 |
| Chocolate mousse | 1 | 2 | 50,0 | 9 | 12 | 75,0 | 0,67 | 0.1605 to 2.7689 | 0.5768 |
| Garlic mayonnaise | 2 | 2 | 100,0 | 8 | 12 | 66,7 | 1,50 | 1.0054 to 2.2379 | 0.0470* |
| Butter | 6 | 9 | 66,7 | 4 | 5 | 80,0 | 0,83 | 0.4408 to 1.5753 | 0.5747 |
| Loin with garlic | 6 | 6 | 100,0 | 4 | 7 | 57,1 | 1,75 | 0.9213 to 3.3240 | 0.0873 |
| Codfish with "Vizcaina"-sauce | 0 | 0 | 0,0 | 10 | 13 | 76,9 | 0,00 | 0.0918 to 4.8440 | 0.6886 |

*These food items had a p value < 0.05. However, since a very low proportion of cases could be explained they were not taken into account.

Supplementary Table 3. Total specimens for laboratory investigation during norovirus GI.5[P4] foodborne outbreak investigation, Murcia, Spain, 2021.

| Sampling subjects | | No. stool samples sent to private laboratory | | No. stool samples sent to MLRRHospital | | No. stool samples sent to CNM | | No. sequences for phylogenetic analysis |
|-------------------|--------------|--|------------------------|--|-----------------|-------------------------------|-----------------------|---|
| | | Processed* | Positive for norovirus | Processed† | Positive for GI | Processed | Positive for GI.5[P4] | |
| | | Food handlers | Symptomatic | 7 | 5 | 6 | 6 | |
| | Asymptomatic | 23 | 8 | 24 | 8 | 8 | 7 | 7 |
| Clients | Symptomatic | 0 | 0 | 3 | 3 | 3 | 3 | 2 |
| Total | | 30 | 13‡ | 33 | 17§ | 16 | 15¶ | 14# |

GI: Genogroup I; MLRRHospital: Microbiology laboratory at Regional Reference Hospital; CNM: National Centre for Microbiology; No.: number; nt: nucleotides

*Private Laboratory used for diagnosis an immunochromatographic test for norovirus/adenovirus/rotavirus Certest Biotec®

†12 out of 13 samples were sent to MLRRHospital.

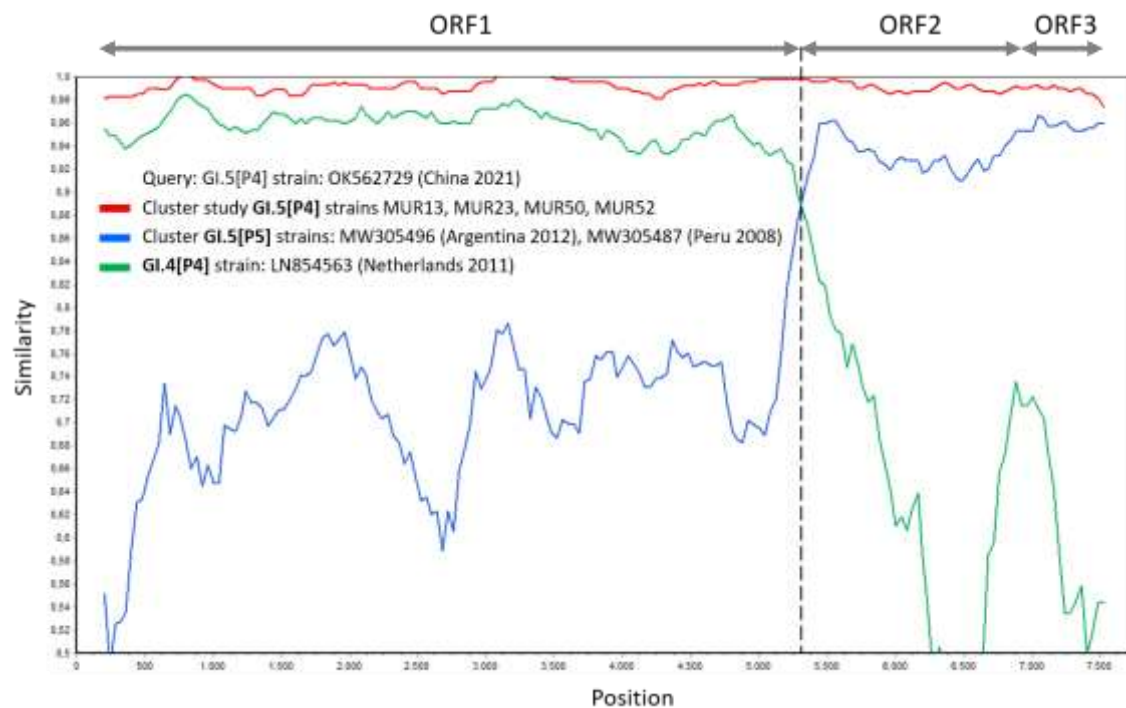
#MLRRHospital used for diagnosis a multiplex one-step real-time PCR platform, the Allplex™ Gastrointestinal Full Panel Assay (Seegene, Seoul, South Korea)

§16 out of 17 samples were sent to CNM. The remaining sample was not sent to CNM because of insufficient stool quantity and therefore remained with no genotyping. The 17 GI-positive samples had a range of cycle threshold values of 23 – 39.4

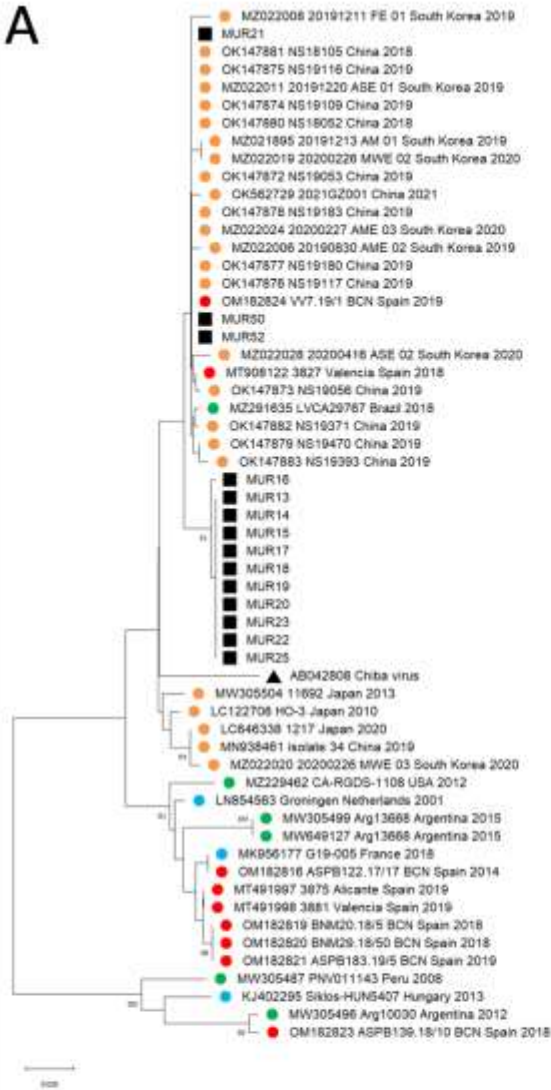
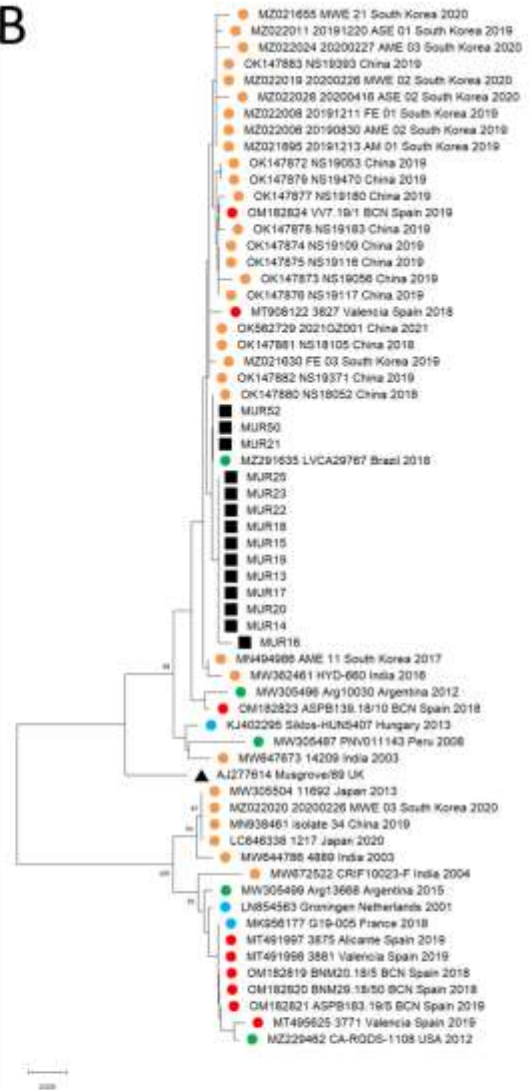
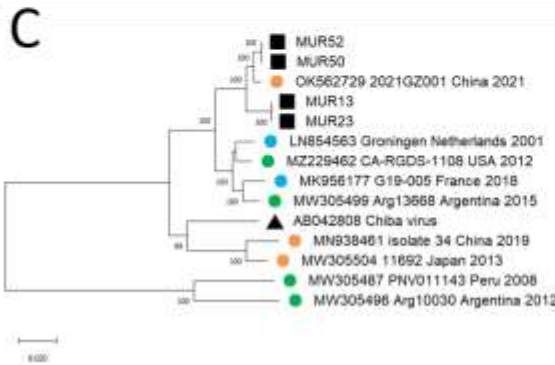
¶15 GI-positive samples with a range of cycle threshold values of 23-35 were successfully amplified by the conventional RT-PCR used for genotyping. The remaining sample had a Ct of 39.4

#Only sequences >450 nt were used for phylogenetic analysis.

Supplementary Figure 1. Plot of similarity of whole-genome nucleotide sequences of GI.5[P4], GI.4[P4] and GI.5[P5] strains from NCBI. The norovirus genetic organization is shown in the top panel. The breakpoint position of recombinant is marked with a dashed line. Analyses were conducted by using SimPlot 3.5.1 (Kimura distance model, window size 400 bp moving in 40 nucleotides steps). We used the only available complete genome of GI.5[P4] strain from China as a query sequence.



Supplementary Figure 2. Phylogenetic analysis of the norovirus genomes identified in this study based on A) 250-bp partial 3'-end of ORF1, B) 274-bp partial 5'-end of ORF2, C) complete ORF1, D) complete ORF2 sequences (Non-collapsed version of Figure 2 with taxa names).

A**B****C****D**