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# Comprehensive Analysis of a Norovirus-Associated Gastroenteritis Outbreak, from the Environment to the Consumer

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**Abstract:**

Noroviruses have been recognized to be the predominant agents of nonbacterial gastroenteritis outbreaks in humans, and their transmission via contaminated shellfish consumption has been demonstrated. Norovirus laboratory experiments, volunteer challenge studies, and community gastroenteritis outbreak investigations have identified human genetic susceptibility factors related to histo-blood group antigen expression. Following a banquet in Brittany, France, in February 2008, gastroenteritis cases were linked to oyster consumption. This study identified an association of the norovirus illnesses with histo-blood group expression, and oyster contamination with norovirus was confirmed by qualitative and quantitative analyses. The secretor phenotype was associated with illness, especially for the non-A subgroup. The study showed that, in addition to accidental climatic events that may lead to oyster contamination, illegal shellfish collection and trading are also risk factors associated with outbreaks.

**Keywords:** oysters, norovirus outbreak, HBGA typing, shellfish outbreak, norovirus quantification.

## INTRODUCTION

Since first identified as the cause of a gastroenteritis outbreak in an elementary school in Norwalk, Ohio, in 1968, noroviruses (NoVs) have come to be recognized as an important agent of non bacterial gastroenteritis in humans (3). NoVs are small non-enveloped viruses containing a single-stranded, positive-sense RNA genome and constitute one of the six genera in the family *Caliciviridae*. Based on genomic sequence and phylogenetic analysis, the NoV genus contains more than 30 genetic types distributed into five genogroups, and they cause infection principally in humans but also in some animals (46). Since the end of the last century, genogroup II (GII) strains have predominated among humans, but numerous strains presenting genomic diversity co-circulate in the population. Many NoV strains bind to histo-blood group antigens (HBGAs) (40). HBGAs are complex glycans present on many cell types including red blood cells and vascular endothelial cells, as well as on the epithelia of the gastrointestinal, uro-genital and respiratory tracts. HBGAs are synthesized from a series of precursor structures by stepwise addition of monosaccharide units via a set of glycosyltransferases. In humans, the pleiotropic interaction of alleles at three loci, *FUT3*, *FUT2* and *ABO* determines the Lewis, Secretor, and ABO phenotypes, respectively (28). Evidence accumulated from volunteers studies and from analysis of outbreaks indicate that binding to these carbohydrates is required for infection (5, 6, 15, 17, 18, 25, 39). Moreover, various human NoV strains that bind to HBGAs present distinct specificities for HBGAs (13, 14, 38). As a result, most strains infect only a subset of the population based on HBGAs expression (9, 24, 40). In addition, some strains of either the GI or GII genogroups were shown to specifically attach to oysters tissues through recognition of histo-blood group antigens (21, 30, 43, 44), suggesting that oysters may act as selective filters, specifically concentrating strains that can recognize carbohydrate epitopes shared with humans.

NoVs infection is characterized by the sudden onset of vomiting or diarrhea or both symptoms (3). Similar to other viruses causing gastroenteritis, NoVs multiply in the intestines and are excreted in large quantities in human feces. Human waste is processed in sewage treatment plants, but the treatment procedures do not completely remove enteric viruses from the water effluents leaving the plant (8, 16). Strains that cause severe symptomatic infection as well as those that cause subclinical infection are excreted into sewage that may then be discharged into coastal environment (11). As these viruses are very resistant to inactivation, the sanitary consequences can include contamination of drinking water, vegetables or bivalve molluscan shellfish (19). Mollusks such as oysters filter large volume of water as part of their feeding activities and are able to accumulate and concentrate different types of pathogens. Regulations based on measuring levels of bacterial enteric pathogens in shellfish tissues (European regulation 54/2004/EC) or in shellfish growing water (United States National Sanitation Program) have been instituted to protect the consumer. However, despite these control measures, outbreaks linked to shellfish consumption still occur after either accidental contamination or incomplete depuration (22, 34, 45). Illegal shellfish collection and trading represent an additional source of food contamination that has received little attention so far. We report here a norovirus outbreak that was due to a breach of such a regulation. In addition, quantitative data of oyster contamination and of consumed oysters in relation with the genetic susceptibility of exposed consumers are reported.

## MATERIALS AND METHODS

**Collection of epidemiological data and statistical analysis.** All data concerning cases of gastroenteritis were collected using a standardized questionnaire that was completed by each participant and addressed foods consumed, symptoms, and timing of illness. Details on patients and the course of the outbreak are presented in the Results section. Association

between food consumption and illness was estimated by calculation of the relative risk (RR) and its 95% confidence interval (CI 95%). Two-by-two comparisons between subgroups were performed using the Fisher' exact test (two-tailed). Analysis of the association between the presence of symptoms and the number of consumed oysters was performed using the Wilcoxon signed ranked test. All statistical analyses were performed using the Prism 5 program (GraphPad Software Inc, La Jolla, CA).

**Analysis of clinical samples**. Five fecal samples collected from five patients were analyzed. Group A rotaviruses (RV), astroviruses (AV) and adenoviruses (AdV) types 40 and 41 were detected as previously described (1). For confirmation, typing and other enteric virus detection, nucleic acids (NA) were extracted and purified using a QIAmp Viral RNA kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Noroviruses genogroup I and II (NoV GI, GII), sapoviruses (SaV), Hepatitis A virus (HAV), enteroviruses (EV), and Aichi viruses (AiV) were detected with several RT-PCR assays that amplified regions previously demonstrated suitable for detection and/or typing (1).

**Analysis of the shellfish samples**. Seven oyster samples (*Crassostrea gigas*) comprising at least 30 oysters per sample (except 18 oysters for # 79) were collected. One sample (# 75) was collected from leftovers at the caterer, three (# 76, 77, 78) from an approved class A production area (less than 230 E.coli/ 100 g of total flesh according to the European Regulation 54/2004/EC) as identified by the REMI Ifremer Surveillance Network, one (# 79) from the same batch in the producer depuration tank, and the last two (# 82, 83) from an area located 30 km from the approved production area and where collection and trading of shellfish is illegal. Shellfish, kept at 4°C during shipment, were analyzed as previously described. Briefly, the stomach and digestive diverticula (DT) were removed by dissection (1.5 g portions), homogenized, extracted with chloroform-butanol, and treated with Cat-floc (Calgon, Ellwood City, PA, USA), and virus was concentrated by polyethylene

glycol 6000 (Sigma, St Quentin, France) precipitation (4). Viral nucleic acids (NA) were extracted using Nuclisens Kit (BioMerieux France), suspended in 100µl of elution buffer with 20 units of RNAse inhibitor (Invitrogen) and analyzed immediately or kept frozen at -80°C (23).

*Real-time RT-PCR***:** All shellfish NA extracts were first screened by real-time RT-PCR (*r*RT-PCR) using previously published primers and probe for NoV, HAV, AV and EV (22). *r*RT-PCR was performed on a MX3000 (Stratagene, France) or an ABI Prism 7000 SDS detector (Applied Biosystem, France), using the Ultrasens® one step Quantitative RT-PCR System (Invitrogen, France). All samples were analyzed in duplicate on 5 µl of undiluted or ten-fold diluted RNA extracts. Two negative amplification controls (water) were included in each amplification series and no more than six samples were analyzed in a *r*RT-PCR assay. Precautions such as isolated rooms for various steps and the usage of filter tips were taken to prevent false positive results.

The cycle threshold (Ct) was defined as the cycle at which a significant increase in fluorescence occurred (i.e. when fluorescence became distinguishable from background). The number of NoV RNA copies present in positive samples was estimated using GI and GII standard curves. To be included in the quantitative analysis, all wells had to yield a Ct value $\leq$ 41. The final concentration was then determined based on the NA volume analyzed (5 µl of 100µl of NA extract) and measured weight of DT (1.5 g analyzed) (23).

The efficiency of virus extraction procedures was determined for each extraction by seeding $10^4$ 50% tissue culture-infective doses of mengovirus prior to sample processing and determining mengovirus recovery by *r*RT-PCR, as previously described (7, 23). NoV concentrations were then corrected for virus loss during extraction by dividing the final norovirus concentration (uncorrected) by the mean mengovirus extraction efficiency.

The presence of RT-PCR inhibitors was evaluated by co-amplification of 2.5 µl of each

NA extract with 2.5 µl containing 100 copies of GI or GII RNA internal controls in separate experiments (23). Amplification of RNA indicated that no more than partial inhibition was present; no adjustments to quantitative estimates were made for samples with partial inhibition.

*Standard RT-PCR*: The viruses that were detected in samples by *r*RT-PCR were typed by sequencing after amplification with a standard, two-step RT-PCR format using 40 cycles of amplification with the same primers as for clinical samples (1, 22).

**Sequence analysis**. Amplicons from virus-positive samples were excised from the gel, extracted and purified for sequencing using a QIAex II gel extraction kit (Qiagen) (1). Sequencing with the BigDye® Terminator Cycle Sequencing Kit (Applera Corporation, Foster City, CA) was performed with the same primers used for amplification (1) . Sequences were analyzed through the European Foodborne Viruses Database (https://hypocrates.rivm.nl/bnwww; FBVE QLK1-CT-1999-00594) for identification of the NoV genotypes. Sequences of other enteric viruses were checked for homologies using the BLAST-search program in GenBank (April-May 2008).

**ABO, secretor and Lewis saliva phenotyping.** Saliva samples were collected from 33 out of 34 individuals who had eaten oysters and immediately stored at -20 °C. They were boiled prior to use in order to remove potentially contaminating virus-specific immunoglobulins as well as bacterial glycosidases. Phenotyping of ABO, secretor and Lewis characters was performed by ELISA as previously described using a set of monoclonal anti-A, B, H and Lewis antibodies and peroxidase-conjugated UEA-I lectin (29).

## RESULTS

**Epidemiological investigation**. In Brittany (France), on February 5, 2008, a banquet was organized for lunch with 80 participants. They were separated in two rooms and oysters

were served in one room only. All sick people had been seated in that room. The menu was a plate with three oysters, three langoustins, and five shrimps, fish and gratin dauphinois, cheese and salad, and fruit tart. As no sign of disease was found among people who did not consumed oysters, the epidemiological study rapidly implicated oysters.

A total of 34 people ate oysters, the mean number of oysters consumed per people was 3.5 (range 2 to 6, 95% CI 3.1- 3.9). Twenty-three people got sick (Fig. 1) with a mean incubation period of 33.4 hours (range 8 to 50 hours, 95% CI 29.0-37.8). Vomiting was reported in 69% of cases and diarrhea in 87%. The duration of symptoms ranged from half a day up to 6 days with a mean of 4.0 days (95% CI, 3.2-4.9). Two people visited a physician. The mean age of the cohort was 43.4 years (range 23 to 60, 95% CI, 39.6-47.2).

**Results of stool analysis**. A total of five stool samples collected from five individual patients were analyzed. All stools were negative for AdV, HAV, RV and EV. No viral pathogen was detected from one stool (E2499). Three stools were positive for NoV GII, one (E2482) of which was also positive for SaV, and a fourth stool (E2455) was positive for AiV (Table 1). After sequencing, a GI.2 strain was identified for the SaV, three GII.4 and a GII.2 for the NoVs and a genotype A for AiV.

**Results of shellfish analysis**. The first sample (# 75) collected on February 13 in the restaurant from the batch that had been consumed, contained GII NoV RNA. The sample collected from the producer (# 79) on February 14, and kept in a depuration tank for 23 days, was also positive for GII NoV as well as for SaV. Neither of these two samples were found to contain AV, AiV, EV, HAV or RV. Three samples collected on February 12 from the producing area, located in South Brittany in a class A area (less than 230 E.coli/ 100 g of total flesh), were negative for all human enteric viruses evaluated. Two additional samples, were collected on February 25 (# 82) and March 5 (# 83) from a distinct location that was

presumptively linked to the outbreak (see discussion). These two samples contained NoV GII, SaV and AV but were negative for AiV, EV, RV and HAV.

Positive samples ( # 75, 79, 82, 83) were extracted four times (except # 79 which was extracted only three times due to the limited number of oysters available) for quantitative analyses. Extraction efficiencies, as measured by recovery of mengovirus seeded into the shellfish prior sample processing, ranged from 12 to 16% for all extracts (Table 2). The measured NoV concentrations for samples # 75 and 79 were quite variable, ranging from negative (one replicate among four for # 75 and one negative among three for # 79) to thousands of RNA copies per gram of DT (Table 2). The two other samples (#82 and 83) showed a more homogenous contamination for all replicates analyzed (all four replicates positive). The geometric mean virus concentrations for all of these samples were quite similar.

**Sequence comparisons.** A short sequence (84 bp) in the NoV polymerase coding region obtained for the sample # 75, corresponded to the GII.4 strain detected in two stool samples (E2457and E2458). The sequence obtained from oyster sample # 82 was identical to that detected in stool E2482 over a 255 bp sequence amplified from the capsid coding region. The sequence from sample # 83 was identified as that of a GII.3 NoV strain (248 bp in the capsid region) and did not match that of any clinical case directly linked to the outbreak but was identical to a secondary case (daughter of E 2455). No virus sequence (NoV or SaV) could be obtained from sample # 79.

**Association between expression of histo-blood group antigens and disease.** ABO, Lewis and secretor phenotypes from 33 individuals who had consumed oysters were obtained from saliva. The phenotyping gave clear-cut results for every case. The frequencies of the various phenotypes in this cohort did not differ from those of the French population (data not

shown). Owing to the small number of Lewis negative individuals (3 among secretors and one among nonsecretors), the potential effect of the Lewis phenotype could not be analyzed. No statistically significant associations were found between the ABO, Secretor and Lewis phenotypes and either the incubation time, the type of symptom reported (vomiting or diarrhea) or the duration of symptoms (data not shown). However, the frequency of individuals reporting illness was lower among nonsecretors than among secretors ($p<0.01$, Fisher's exact test). When evaluated by symptom, both vomiting and nausea were significantly less frequent in the nonsecretor group than in the secretor group. To analyse the effect of the ABO phenotype, the secretor group was spilt between A, B and O subgroups. A direct comparison of these three subgroups did not show any significant difference between them. However, when compared to the nonsecretor group, only the B and O secretor groups showed a significantly higher frequency of illness (Table 3). The A secretor subgroup did not statistically differ from the nonsecretor group, indicating that most of the effect of the secretor phenotype was borne by the non A subgroups (O and B). This suggests that epithelial expression of the A blood group may have hindered recognition of the carbohydrate NoV receptor and comparison of the A secretor group to the non A secretor group (B+O) indeed showed that among secretors, A blood group individuals were less likely to have diarrhea ($p<0.05$). This suggest that the A subgroup was not as sensitive as the non-A subgroup though the low number of patients made it difficult to reach statistical significance.

Since the age of subjects and the number of consumed oysters could be possible confounding factors, we verified that there was no association between any of the histo-blood group phenotype and these two parameters (data not shown). When considering subjective symptoms such as nausea and abdominal pain in addition to vomiting and diarrhea, more individuals of the cohort were affected. Interestingly, within the group of six nonsecretor individuals, the number of consumed oysters was higher among the three who reported at

least one symptom (p=0,026, Wilcoxon signed rank test), suggesting that nonsecretors could not become infected below a certain virus dose threshold. Possibly these nonspecific symptoms of nonsecretors were due to infection with viruses other than norovirus. Alternatively, they could be due to the ability of the norovirus strain involved to show some cross-reactivity for carbohydrate motifs shared between secretors and nonsecretors as previously observed for some strains (24, 41). Regardless, collectively these results indicate that the nonsecretor phenotype was a protective factor and that within the secretor group, blood group A was also protective.

## DISCUSSION

The institution of regulations to specify acceptable levels of bacterial enteric pathogens in shellfish tissues (European regulation,54/2004/EC) or in shellfish growing waters (United States National Shellfish Sanitation Program) led to the classification of production areas. In addition to mandatory controls performed by producers, in France, Ifremer has set up a surveillance network to control shellfish quality (REMI) and to collect data from environmental events. In the past this allowed the identification and evaluation of contamination events associated with oyster-related outbreaks (20, 22). In the outbreak described here the producing area was located in South Brittany in a class A area and no environmental event such as rain fall or sewage treatment plant failure was reported in previous months. This was confirmed by the absence of viral contamination in the samples collected for the REMI controls on February 12. It was thus surprising to observe that both samples related to a specific producer from that clean class A area were clearly contaminated by viruses and were the cause of clinical cases. A few days later, police arrested a fisherman collecting oysters illegally from a forbidden area located in a major harbour. After a day in jail, the man admitted selling these illegal oysters to the producer implicated in the outbreak.

This was confirmed by sequencing of the NoV strains detected in the sample taken by the police (# 82) and in the forbidden area (# 83). This outbreak therefore clearly illustrates the danger of breaching regulations and refusing to consider the usefulness of the producing area classification as some producers do.

The direct detection of viral human pathogens has became the most reliable manner for documenting viral contamination of shellfish, and sensitive methods are now available (4, 7, 23). Since the development of molecular methods, the inclusion of quality controls became a priority and was facilitated by the use of real time RT-PCR allowing sample to sample comparisons. Mengovirus (mengovirus strain $MC_0$, *Picornaviridae* family member) was previously shown to be suitable as an external control for extraction efficiency based on structural characteristics and absence from environmental samples (7, 23). Using the different controls, the method allowed us to be confident that concentrations expressed here reflect correctly the amount of viruses present in the contaminated oysters. No adjustments for PCR amplification efficiency were made since controls showed no effect of nucleic acid extracts on *r*RT-PCR performance (data not shown). The last point that may influence NoV quantification is sequence variation in different NoV genotypes leading to mismatches with the primers used in the real-time assay. This concern was offset by the results of previous studies that demonstrated the broad reactivity of the NoV-specific primers and probes used in the current study and by the fact that the predominant strain detected here is a GII.4 corresponding to the strain used to build the standard curve (8). The sample concentration expressed as a minimum and a maximum level (defined by taking account extraction efficiency), is one approach to estimate NoV levels of contamination. The large amount of oysters available allowed us to extract each sample at least four times to evaluate the reliability of our quantitative approach. The sample directly implicated in the outbreak showed the largest variation for NoV concentrations (up to 1000-fold). The high level of

variability may be explained by the producer's information who later recognized having mixed several batches of oysters. After almost one month in depuration tank the geometric mean concentration had decreased only approximately two-fold, although variability within replicates was lower. This is another demonstration that virus contamination decreases very slowly within shellfish tissues and that the use of depuration tanks, although efficient in case of bacterial contamination, has little utility in addressing viral contamination (22, 27, 35). The measured levels of virus contamination in the two samples made up of shellfish entirely originating from the forbidden area were more homogenous, suggesting a frequent exposure to fecal contamination, possibly due to the close proximity from a major harbor.

The level of NoV contamination in the present report was only slightly higher than the levels of viral shellfish contamination previously described for NoV outbreaks (20, 22, 36), or for an HAV outbreak (7). Although quantitative information on the levels of NoV contamination in shellfish implicated in outbreaks is still limited, it is well above minimal infectious doses estimated from volunteers studies (2, 42).

Evidence accumulated over the past six years indicates that HBGAs serve as ligands for NoV infection (5, 6, 15, 17, 18, 25, 26, 39). However some studies showed discrepant results concerning the effect of either the ABO or the secretor phenotype, raising questions about the importance of HBGAs in norovirus infection (10, 12, 31, 32, 37). These discrepancies could be due to several factors, including ill-defined carbohydrate specificity of the causative strain and poor or incomplete phenotyping of the affected peoples. The latter aspect is particularly relevant when variable or no associations with the ABO phenotype were reported. Since distinct strains show different specificities for HBGAs, variable effects of the ABO phenotype are expected to occur in outbreaks caused by different NoV strains. Likewise, since expression of ABH antigens in the gut surface epithelial cells is strictly dependent upon the secretor status (33), a lack of information on the secretor phenotype

makes it difficult to observe associations between HBGAs expression and infection by NoVs. In the present study, we observed that the secretor phenotype was a risk factor associated with illness. That was particularly clear when considering individuals of the non A subgroup, indicating that the A blood group antigen, which can be expressed on digestive epithelial cells of secretors only, may have been a protective factor in this particular outbreak. Thus, polymorphisms at both the *FUT2* and *ABO* loci controlled sensitivity to disease in this shellfish-related outbreak, confirming the importance of these polymorphisms in determining susceptibility to NoV infection previously observed from either volunteer studies or communities outbreaks (2, 5, 17, 24, 25). In this study, several strains (NoV, AiV, SaV) were detected either in stool samples or in shellfish. However, NoV genetic sensitivity seems to be predominant, suggesting that they induced most illnesses.

The comprehensive approach of our study, consisting of food analysis, viral quantification and HBGA typing of consumers is novel in examination of food safety and provides a new approach for food safety analysis. The importance of the presence of different enteric viruses in stool and shellfish samples raises questions about which viruses were responsible for illness. The impact of HBGA expression in individuals with illness clearly implicates the GII NoVs as the predominant causative agents of the outbreak. When multiple enteric viruses contaminate a food product, genetic analysis of affected individuals can complement data collected from clinical and environmental samples and may provide important information for risk analysis and future food safety regulation.

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TABLE 1: Patient symptoms and viruses detected from stool samples.

| Stool sample | Saliva phenotype | Clinical signs | | Virus detected | Oysters consumed[a] |
|---|---|---|---|---|---|
| | | vomiting | diarrhea | | |
| E2455 | SEC, B, Le+ | + | + | AiV | 6 |
| E2457 | SEC, B, Le+ | + | + | NoV GII.4[b] | 3 |
| E2458 | SEC, O, Le+ | + | + | NoV GII.4 | 3 |
| E2482 | *No saliva* | + | + | NoV GII.4, GII.2, SaV GI.2 | 3 |
| E2499 | No sec, Le+ | - | + | none | 6 |

[a] number of oysters consumed during the lunch,

[b]GII.4 underlined identifies strains with identical sequences detected in the oyster samples.

TABLE 2: Detection and quantification of NoV in oyster samples.

| Sample | Date | Mean extraction efficiency (%)[a] | Number of Positive Replicates/Number analyzed | Genogroup II NoV geometric mean concentration (range)[b] | |
|---|---|---|---|---|---|
| | | | | uncorrected | corrected |
| Caterer (#75) | 02/13 | 16.1 | 3/4 | 229 (48-2548) | 1727 (89- 23162) |
| Producer (#79) | 02/14 | 13.7 | 2/3 | 144 (63- 273) | 957 (476- 2054) |
| Harvested illegally (#82) | 02/25 | 12.9 | 4/4 | 301 (35- 913) | 2361 (273- 7509) |
| Forbidden area (#83) | 03/05 | 12.6 | 4/4 | 776 (273-1885) | 6076 (211-14839) |

[a]Mean percentage of mengovirus RNA copy number recovered in shellfish extracts relative to the amount of mengovirus added to samples prior to extraction

[b]Geometric mean concentration of positive samples expressed in RNA copies per g of DT, (range of concentrations detected), before (uncorrected) and after (corrected) correction for the mean extraction efficiency

TABLE 3: Effect of the blood group phenotype on illness and symptom frequency

| HBGA | nonsec (N= 6) | Sec (N= 27) | A Sec (N=10) | B Sec (N=4) | O Sec (N=12) | non A Sec (N= 16) |
|---|---|---|---|---|---|---|
| Illness[a] | 1[b] | 21[c] | 6 | 4[d] | 10[e] | 14[f] |
| Vomiting | 0 | 15[d] | 4 | 4[f] | 7[d] | 11[e] |
| Diarrhea | 1 | 18[e] | 4 | 4[d] | 9[d] | 13[e] |

[a]Total number of individuals in each group

[b]Individuals who had either vomiting or diarrhea.

[c,d,e,f]Comparisons by two tailed Fisher's exact test between the nonsecretor (nonsec) group and either the secretor (Sec) group or the secretor group split by A, B and O phenotypes: A Sec, B Sec, O Sec, non A Sec (O or B Sec phenotypes). A single AB blood group secretor individual with diarrhea was not included in the analysis of A or B phenotypes. [d]$p<0.05$, [e]$p<0.02$, [c]$p<0.01$, [f]$p<0.005$.

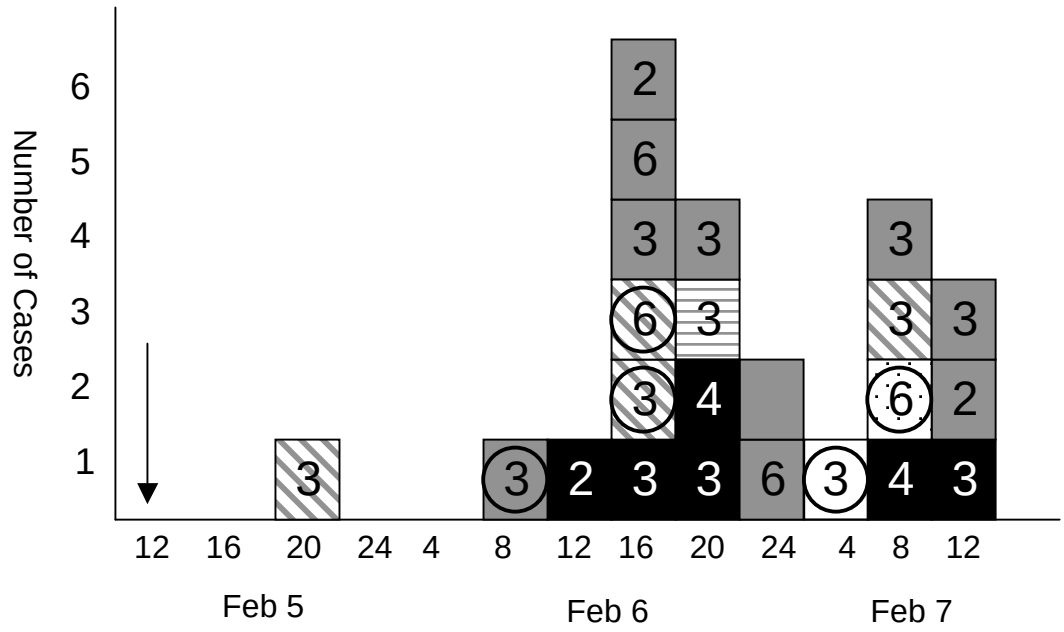
FIGURE LEGEND

Figure 1: Onset of symptoms for banquet participants.

Oysters were consumed on February 5 for lunch (arrow). Each box represents one new clinical case identified in 4 hour intervals (x axis), and the number of cases is recorded (y axis). The number in each box corresponds to the number of consumed oysters, and the circle identify peoples from whom stool was collected for analysis.

Black box: type A secretor, grey: type O secretor, horizontal stripe: type AB secretor, diagonal stripe: type B secretor, and dots: non secretor individual. The empty box: no saliva collected.

1


Number of Cases
6
5
4
3
2
1
12
16
20
24
4
8
12
16
20
24
4
8
12
Feb 5
Feb 6
Feb 7