

## TRANSMISSION OF NORWALK VIRUS DURING A FOOTBALL GAME

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**ABSTRACT**

**Background** During a college football game in Florida, diarrhea and vomiting developed in many of the members of a North Carolina team. The next day, similar symptoms developed in some of the players on the opposing team.

**Methods** We interviewed those who ate the five meals served to the North Carolina team before the game and some of the players on the opposing team who became ill. Patients with primary cases were members or staff of the team who had vomiting or diarrhea at least 10 hours after but no more than 50 hours after eating a box lunch served the day before the game. Patients with secondary cases had a later onset of symptoms or had symptoms without having eaten the box lunch. Stool samples were examined by electron microscopy and by a reverse-transcription-polymerase-chain-reaction (RT-PCR) assay.

**Results** The two football teams shared no food or beverages and had no contact off the playing field. Of five meals served to the North Carolina team before the game, only the box lunch was associated with a significant risk of illness (relative risk of illness, 4.1; 95 percent confidence interval, 1.6 to 10.0). The rate of attack among those who ate the box lunch was 62 percent. There were 11 secondary cases among the members and staff of the North Carolina team and 11 such cases among the Florida players. All four stool samples obtained from North Carolina patients were positive for Norwalk-like virus on electron microscopy. All four samples as well as one of two stool samples from players on the Florida team were positive for a Norwalk-like virus of genogroup I on RT-PCR assay; the RT-PCR products had identical sequences.

**Conclusions** This investigation documents person-to-person transmission of Norwalk virus among players during a football game. Persons with acute gastroenteritis should be excluded from playing contact sports. (N Engl J Med 2000;343:1223-7.)

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**N**ORWALK-LIKE viruses, which are small (27 to 32 nm), round viruses that belong to the family Calciviridae, cause 96 percent of the outbreaks of acute nonbacterial gastroenteritis in the United States.<sup>1-4</sup> The degree to which infection with Norwalk-like virus is endemic is unknown because most of those affected do not seek medical care for acute gastroenteritis. The development of molecular methods to detect and differentiate Norwalk-like viruses has increased the opportunity to combine laboratory and epidemiologic evidence to document the relatedness of cases in an outbreak.<sup>5</sup>

On Friday, September 18, 1998, after a locker-room lunch, the members and the staff of a North Carolina college football team flew to Florida for a 7 p.m. game the next evening. During the football game, several players on the North Carolina team suddenly began to vomit and have diarrhea. They continued to play despite their illness. The nature of the game made it difficult for players to avoid contact with feces and vomitus. Other members of the North Carolina team and staff who did not travel to Florida also became ill. By Sunday evening, some of the Florida players also had similar gastrointestinal symptoms. The only contact between the teams had been on the playing field. We conducted an investigation to determine the cause of this gastrointestinal illness, the source of infection, and the mode of transmission.

**METHODS****Epidemiologic Investigation**

We conducted a retrospective cohort study to examine risk factors for diarrhea or vomiting. Primary cases were initially defined by the occurrence of vomiting or diarrhea among members, staff, or associates of the North Carolina football team. After identifying the meal responsible — a box lunch served the day before the game — and the organism responsible, we refined the definition to include any instance of vomiting or diarrhea among such persons at least 10 hours after but no more than 50 hours after the consumption of the box lunch. This adjustment was made on the basis of the incubation period for illness caused by Norwalk-like virus, which can range from 10 to 50 hours.<sup>6</sup> Secondary cases were defined by the occurrence of vomiting or diarrhea among members, staff, or associates of either football team who had not eaten the meal or who began to have symptoms more than 50 hours after eating the meal.

**Laboratory Investigation**

Four stool samples from North Carolina patients and two from Florida patients were collected within 24 hours after the onset of diarrhea. The samples were cultured for salmonella, shigella, and campylobacter species. Stool samples were examined by electron microscopy according to standard methods<sup>7</sup> and tested for Norwalk-like virus RNA by a reverse-transcription-polymerase-chain-reaction (RT-PCR) assay with the use of broadly reactive G1 and G2 primers for conserved regions of the RNA polymerase gene according to previously described methods.<sup>8</sup> Viral RNA was released from virus particles in a 20 percent stool suspension with use of a modified heat-release method<sup>9</sup> or extracted with use of a polyethylene glycol-cetyltrimethylammonium bromide precipitation protocol.<sup>10</sup> Positive and negative controls were included in the RNA extrac-

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tion and RT-PCR assay. The positive control for genogroup I Norwalk-like viruses was a stool specimen from a person voluntarily infected with Norwalk virus, and the positive control for genogroup II was a stool specimen obtained during an outbreak of infection with a genogroup II Norwalk-like virus. Negative controls contained equal volumes of water in place of RNA. The RT-PCR products were sequenced to determine the relatedness of the strains from the North Carolina and Florida patients. IgG antibody against recombinant Norwalk virus antigen was measured in serum samples obtained during the acute illness and during convalescence with use of an enzyme-linked immunosorbent assay.<sup>11</sup>

Food was collected from a remaining box lunch at the restaurant where it had been prepared and was tested for Norwalk-like virus with a RT-PCR assay. Southern hybridization was used to increase the sensitivity of detection (Boehringer Mannheim).

### Environmental Inspection

Environmental health specialists inspected the restaurant where the box lunches eaten by the team had been prepared. Food-processing procedures were reviewed, and food-storage temperatures were checked on all refrigeration units.

### Statistical Analysis

Chi-square tests for statistical significance were carried out with use of Epi Info software.<sup>12</sup> Multivariate analysis was performed with use of stepwise, backward logistic regression (SAS software, SAS Institute) to identify the specific foods associated with illness.

## RESULTS

### Epidemiologic Investigation

We interviewed 108 members of the North Carolina team and support staff (including 66 team members) who shared at least one of the five meals served, 8 of the 11 Florida players who became ill with vomiting or diarrhea after the game, and 2 food handlers (1 of whom owned the restaurant) who had prepared the box lunches for the North Carolina team. Of the members and staff of the North Carolina team whom we interviewed, 65 of the players and 36 of the support staff had traveled by private plane to Florida on Friday, September 18, 1998. The first group meal, eaten in North Carolina, was a catered box lunch served in the athletic facility on Friday, whereas the four meals eaten in Florida were breakfast, lunch, and two dinner buffets served by one hotel kitchen, exclusively for the North Carolina team. The Florida players and the food handlers who were interviewed said they had not eaten any of the meals shared by the members and staff of the North Carolina team; the members of the opposing teams also did not share drinking water on the field or in the field house.

We identified 54 persons with illness among the 108 persons from North Carolina who were interviewed (50 percent). Forty-three of the patients met the definition for a primary case, and 11 were defined as having secondary cases. Seven of the 108 persons who were interviewed did not travel to Florida; of these 7, 4 met the definition for a primary case, and 2 were defined as having secondary cases. Table 1 illustrates the rate of illness among members of the North Carolina and Florida teams according to their playing position. Of the 36 North Carolina players who were defined

**TABLE 1. RATE OF ILLNESS AMONG FOOTBALL PLAYERS ACCORDING TO THEIR POSITION AND TEAM.**

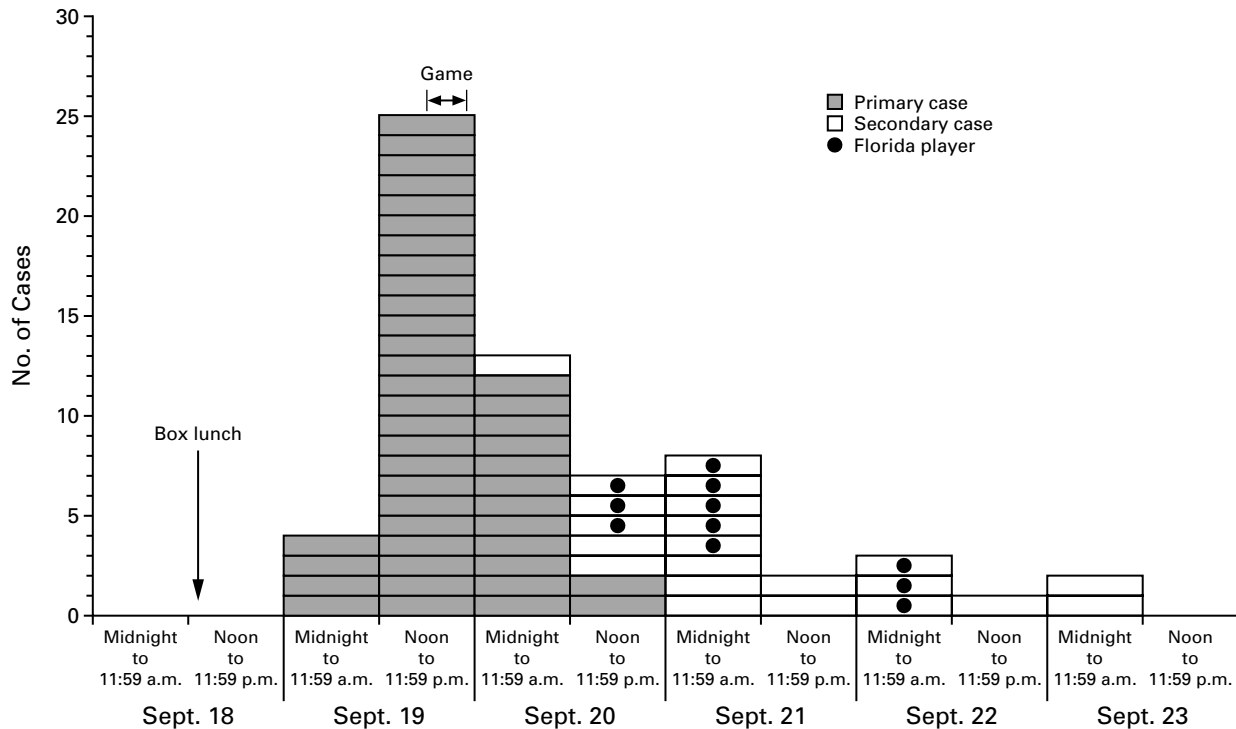
POSITION	NORTH CAROLINA TEAM		FLORIDA TEAM	
	TOTAL NO.	NO. WHO BECAME ILL (%)	TOTAL NO.	NO. WHO BECAME ILL (%)
Offense	35	22 (63)	34	11 (32)
Defense	30	14 (47)	30	0
Total	65	36 (55)	64	11 (17)

as having primary or secondary cases, 19 (53 percent) began to have vomiting, diarrhea, or both before or during the game. The 18 patients from North Carolina who were not football players included coaches, trainers, and team physicians.

Of the total of 65 patients, 62 provided complete information on their symptoms: 95 percent reported diarrhea, 90 percent nausea, 76 percent abdominal cramps, 58 percent vomiting, 71 percent headache, and 47 percent fever. The median incubation period for the primary cases was 37 hours (Fig. 1), and the median duration of illness was 36 hours. Of the five meals served between noon on Friday, September 18, and late afternoon on Saturday, September 19, only the lunch served on September 18 in North Carolina was significantly associated with illness among persons from North Carolina, with a rate of illness of 62 percent (Table 2). Univariate analysis showed that all three items in the meal were significantly associated with illness (Table 3). These items were all served together in the same box. Using multivariate logistic-regression analysis to adjust for each of the other items in the box lunch, we found that the turkey sandwich was the only item whose consumption was significantly associated with illness (odds ratio, 4.9; 95 percent confidence interval, 1.3 to 18.9) (Table 3).

### Laboratory Investigation

No bacterial pathogens were isolated from the six stool samples that were tested. In the four samples obtained from North Carolina patients, electron microscopy revealed small, round virus particles whose appearance was consistent with that of a Norwalk-like virus. All four of these stool samples as well as one of the two from Florida patients were positive for genogroup I Norwalk-like virus by a RT-PCR assay. RT-PCR products from two North Carolina patients and one Florida patient were sequenced and compared with each other and with reported sequences in GenBank. All three sequences were identical and had the greatest degree of homology (85 percent of the 81 nucleotides were identical) with the Thistle Hall 1/91 strain from the United Kingdom. Other GenBank sequences with close homology to the strain in the



**Figure 1.** Time of Onset of Illness among Members and Staff of a North Carolina Football Team and Members of a Florida Football Team, September 18 through 23, 1998.

A primary case was defined as any instance of vomiting or diarrhea among members, staff, or associates of the North Carolina football team at least 10 hours after but no more than 50 hours after the consumption of the box lunch. A secondary case was defined as the occurrence of vomiting or diarrhea among members, staff, or associates of either team who had not eaten the box lunch or who began to have symptoms more than 50 hours after eating the box lunch.

current outbreak were from the United Kingdom, the Netherlands, and Norway.

Paired serum samples from two of three patients from North Carolina (including the restaurant owner, who prepared the turkey sandwiches) showed that the IgG titer more than quadrupled between the acute phase and the convalescent phase of the illness. The restaurant owner and an assistant food handler who prepared the box lunches denied having had any diarrhea recently. No virus was detected in food samples from the box lunch.

**Environmental Inspection**

Inspection of the restaurant revealed problems with food handling and preparation. A single sink was used for hand washing, as well as washing utensils, pots and pans, and vegetables. The temperature of items in the refrigerator exceeded the maximal recommended temperature of 7.2°C (45°F). In addition, the slicer had not been sanitized between its use on the tomatoes and its use on the turkey included in the sandwiches.

**DISCUSSION**

We investigated an outbreak of gastrointestinal illness in two college football teams that was caused by

**TABLE 2.** RATE AND RISK OF ILLNESS AMONG MEMBERS OF THE NORTH CAROLINA FOOTBALL TEAM, ACCORDING TO THE MEALS EATEN ON SEPTEMBER 18 AND 19, 1998.

MEAL	DATE	ATE MEAL		DID NOT EAT MEAL		RELATIVE RISK (95% CI)*
		TOTAL NO.	NO. WHO BECAME ILL (%)	TOTAL NO.	NO. WHO BECAME ILL (%)	
Lunch	9/18	81	50 (62)	27	4 (15)	4.1 (1.6-10.0)
Dinner	9/18	87	45 (52)	21	9 (43)	1.2 (0.7-2.2)
Late dinner	9/18	63	34 (54)	45	20 (44)	1.2 (0.8-1.8)
Breakfast	9/19	85	42 (49)	23	12 (52)	0.9 (0.6-1.5)
Lunch	9/19	76	39 (51)	32	15 (47)	1.1 (0.7-1.7)

\*CI denotes confidence interval.

a Norwalk-like virus of genogroup I. In this outbreak, transmission of the virus from a turkey sandwich in a box lunch accounted for 95 percent (41 of 43) of the primary cases. The fact that no virus was isolated from samples of these sandwiches does not discredit the epidemiologic evidence, because methods to detect Norwalk-like viruses in foods are still being refined. The

**TABLE 3.** FOOD-SPECIFIC RATES AND RISKS OF ILLNESS AMONG PERSONS WHO ATE COMPONENTS OF THE BOX LUNCH ON SEPTEMBER 18, 1998.\*

FOOD	ATE LUNCH		DID NOT EAT LUNCH		UNADJUSTED ODDS RATIO (95% CI)	ADJUSTED ODDS RATIO (95% CI)
	TOTAL NO.	NO. WHO BECAME ILL (%)	TOTAL NO.	NO. WHO BECAME ILL (%)		
Sandwich	63	45 (71)	18	5 (28)	2.6 (1.2–5.5)	4.9 (1.3–18.9)
Apple	36	28 (78)	45	22 (49)	1.6 (1.1–2.3)	2.4 (0.6–9.3)
Candy bar	63	43 (68)	18	7 (39)	1.8 (1.0–3.2)	1.6 (0.5–5.0)

\*The adjusted odds ratios were adjusted for each of the other foods in the box lunch. CI denotes confidence interval.

isolation of a Norwalk-like virus from contaminated delicatessen foods linked to a food handler was documented only recently.<sup>13</sup> Evidence from other outbreaks of infection caused by Norwalk-like viruses<sup>14</sup> and from studies in humans<sup>15</sup> indicates that the infectious dose is extremely low. A low level of viral contamination in the food could be sufficient to cause an outbreak and yet be insufficient to be detected by microbiologic analyses of food samples.

Our serologic data suggest that the turkey sandwiches may have become contaminated by an infected food handler. The two persons who prepared the box lunches had no reported history of illness, but one had a clinically significant rise in the titer of antibody against recombinant Norwalk virus antigen, indicating the occurrence of a recent infection with a Norwalk-like virus. The sandwich ingredients could also have been contaminated from another source in the kitchen. A previous outbreak of Norwalk-like virus infection was linked to the consumption of a tossed salad that may have been cross-contaminated by raw shellfish, which had been rinsed in a sink that was later used to separate lettuce.<sup>16</sup> This type of contamination might have occurred in the outbreak we describe, because the lettuce and tomatoes used for the sandwiches were washed in the same sink that was used for other foods, hands, utensils, and pots and pans. The lettuce, tomato, or turkey may also have been contaminated with the virus before the lunch was prepared, since pathogens can remain on lettuce and tomatoes despite their having been rinsed with water.<sup>17</sup>

The hypothesis that a Norwalk-like virus, in effect, crossed the scrimmage line by means of person-to-person transmission on the football field is supported by reports from the team physician and coaches that players were retching on the sidelines as well as in the locker room and that some were playing in uniforms soiled with vomitus and feces. Both fecal-oral transmission and aerosol transmission of vomitus probably occurred, given the intense physical contact and use of bare hands that are characteristic of the game of football.

Several members of the opposing team had an illness compatible with a Norwalk-like virus infection, and the RNA sequence of the virus isolated from a stool specimen from one Florida player was identical to the RNA sequence of virus isolated from the stool specimens from four North Carolina patients. It is unlikely that this Florida patient had been infected with the identical strain of virus from another source, given his history of exposure and the fact that genogroup I strains are uncommon in the United States. Most recent outbreaks of Norwalk-like virus infection in the United States have been associated with genogroup II,<sup>4</sup> whereas the strain detected in this outbreak was more closely related to recently identified European strains.

The finding that both offensive and defensive players from the North Carolina team became ill was not unexpected, because all of these players had a similar risk of exposure to the implicated meal; however, we have no clear explanation for the disparity in the rates of illness between the offensive and defensive players on the Florida team. Although the Florida team won the game by a large margin (final score, 62 vs. 13), each team had possession of the ball for approximately equal lengths of time (29 minutes in the case of the Florida team and 31 minutes in the case of the North Carolina team). Hence, the offensive and defensive units of the Florida team had similar degrees of exposure to ill North Carolina players, although some of the ill defensive players on the North Carolina team may have been more efficient transmitters of the virus than their teammates on the offensive team. This possibility is supported by the observation that a number of the defensive players had vomiting and diarrhea during the game and that these players made most of the tackles of the Florida offense.

Numerous outbreaks of infectious disease associated with sporting events or teams have been described.<sup>18</sup> Previous reported outbreaks of enteric-virus infections among sports participants were caused by the sharing of contaminated water or ice.<sup>19</sup> The only previous reports of person-to-person transmission during foot-

ball games were three outbreaks of *Staphylococcus aureus* infections.<sup>18</sup> Other reports of outbreaks of Norwalk-like virus infection have suggested that in cases in which there is lack of evidence of direct contact with feces, the infection may have been transmitted by aerosolized vomitus.<sup>20,21</sup> The detection of small, round virus particles in vomitus suggests that exposure to vomitus may pose a risk of illness in susceptible persons.<sup>22</sup>

We documented person-to-person transmission of an enteric virus among players during a sporting event by linking classic and molecular epidemiologic methods. Clusters of disease can also be differentiated on the basis of molecular evidence. Two weeks after this outbreak, Norwalk-like virus infections occurred in a fraternity house on the Florida campus where the football game was played (Krause G: personal communication). The infection was thought to have been transmitted by the football players, but a different viral strain was identified. Sequencing data indicated that, despite the geographic and temporal similarities, these two events had different causes.

Our study was limited by several factors. In outbreaks of Norwalk-like virus infection, the onset of secondary illness often overlaps the late onset of primary cases, so that some primary and secondary cases may have been misclassified. In addition, because of the mild nature of some Norwalk-like virus infections, the full extent of this outbreak was probably not recognized. Mild, inapparent, and self-limiting infections may have gone undetected, resulting in underreporting of cases.

On the basis of our findings, we recommend that players with acute gastroenteritis be excluded from competition in order to avoid transmitting the disease to other players. Person-to-person transmission is second to foodborne transmission as the most common method of the spread of Norwalk-like viruses,<sup>4</sup> underscoring the importance of educating players and support staff on appropriate hygiene measures, such as hand washing.

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