Transient Increase in Diarrheal Diseases after the Devastating Earthquake in Kocaeli, Turkey: Results of an Infectious Disease Surveillance Study

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Immediately after the devastating earthquake in Turkey in August 1999, an infectious disease surveillance system was established in Kocaeli Province (the biggest area affected). This surveillance study was mainly focused on diarrheal diseases. During a 33-day period, 1,468 stool cultures were processed. Diarrheal diseases increased step-by-step and later decreased to the initial level by the end of this period. Cases were scattered throughout the entire region, and the identified causes were various, indicating a multifocal increase. Of the identified causes, Shigella species were the most common. Nevertheless, Shigella isolates also belonged to distinct serotypes and clones. This study indicated a multifocal, multiclonal increase in diarrheal diseases after this massive disaster, thus indicating the necessity to set up infectious disease surveillance systems after such events.

Earthquakes are among the most important natural disasters that cause mass emergencies. Depending on the nature and location of such disasters, various infections have caused problems in the past [1-4].

The earthquake on 17 August 1999 in Turkey affected an area 150 km in length. As a consequence, >16,000 people died, and 500,000 were left homeless. Kocaeli Province was the biggest area affected (approximately two-thirds of all areas affected were in Kocaeli Province). The infrastructure of the region, including the water supply system, was heavily damaged. The survivors, on the other hand, were under the stress of losing their relatives, friends, and possessions. Furthermore, the sudden change in the food and living conditions complicated the defense of the population against infectious diseases. The circumstances for the spreading of infectious agents were more suitable than ever. On the basis of these circumstances, a surveillance system to detect and control infectious disease outbreaks was established immediately after the earthquake in Kocaeli Province. Here, we present the results and the lessons derived from this infectious disease surveillance study.

Materials and Methods

Surveillance study design. The surveillance and outbreak control plan was based on 3 parameters: the most probable areas (risk zones), the most probable way of spreading infections, and the most probable etiologic agents.

We divided Kocaeli Province into 4 zones: zone A, zone B, the central zone, and peripheral areas. Zones A and B were the opposite sides of the Gulf of Izmit. The central zone was Centrum and the closest area to the city’s center. All other towns and villages were peripheral areas. Zones A and B were facing the center of the earthquake on the opposite shores of the Gulf of Izmit; therefore, these areas had heavy damage to the buildings and the infrastructure. The central zone, on the other hand, was the area where the damage was relatively less. However, the aftershocks continued, and a huge number of people in this area were living in tents. Peripheral areas had the least damage; therefore, there was less of a threat for an outbreak in these locations.

Because of the hot summer, the damaged infrastructure, and the difficulties of obtaining safe water, we assumed that waterborne diseases were the primary disease threats. Microorganisms that spread by water and that were seen before the earthquake, therefore, could be the probable causes of outbreaks. These microorganisms were Shigella species, Salmonella species, and Giardia intestinalis.

The surveillance system was composed of a laboratory, an infectious diseases ward, and ambulance teams. In the laboratory, a computer was reserved for this study. A database based on the questionnaire used in the surveillance study was prepared.

Diarrhea was defined as ≥3 watery stools per day. Separate protocols of diarrheal diseases for the first-step health organizations (which were located at zones where the risk for outbreaks was high) and for the surveillance center were prepared. Ambulance teams distributed transport media and study protocols to the first-step health organizations and visited them, as well as the tent camps located at those regions, daily. The aim was to refer the stool samples or the patients to the surveillance center.

Microbiology laboratory studies. A microbiology laboratory was established, to identify common bacterial and protozoan agents causing waterborne infectious diseases. Samples were obtained either as stool samples in special containers or as rectal swabs. Stool samples were processed immediately in 2 steps: first, the samples were cultivated on blood agar, MacConkey agar, sal-
monella-shigella agar, and thiosulfate citrate bile salts sucrose agar; second, direct microscopic examination was performed. All media were ready to use (EKBAK, Istanbul, Turkey). MacConkey agar and salmonella-shigella agar were used for the detection of *Salmonella* and *Shigella* species, whereas thiosulfate citrate bile salts sucrose agar and blood agar were used for the detection of *Vibrio* species. Plates were incubated for $\geq 18$ hours at 37°C. Suspected colonies were subcultured onto triple sugar iron agar and blood agar. After incubation for 18 hours at 37°C, non-lactose-fermenting bacteria were tested for agglutination with antisera specific to *Shigella* species, *Salmonella* species, and *Vibrio cholerae* (Difco Laboratories, Detroit).

Direct microscopic examination documented the number of leukocytes per high-power field. Although protozoa that could be readily identified by direct examination were included in the detection analysis, those needing special staining techniques, such as *Entamoeba histolytica*, were omitted from this analysis.

The aim of the microbiology laboratory and the study protocol was to detect the most probable microorganisms. More sophisticated methods, protocols, and techniques were not applicable under these conditions.

**Antibiotic susceptibility tests and DNA studies.** Agar dilution tests for antibiotic susceptibility were performed on Mueller-Hinton agar (Oxoid, Oxoid, Basingstoke, United Kingdom) according to the recommendations of the National Committee for Clinical Laboratory Standards [5]. In brief, $\sim 10^5$ cfu of bacteria per spot were inoculated onto freshly prepared Mueller-Hinton agar plates containing serial 2-fold dilutions of the related antibiotics by means of a multipoint inoculator; agar plates were evaluated after 18 hours of incubation at 37°C, and the MICs were determined. Powder forms of the following antibiotics were used: ceftazidime (Glaxo-Wellcome, Istanbul); ampicillin (Pfizer, Istanbul); ciprofloxacin (Bayer, Istanbul); gentamicin, tetracycline, and chloramphenicol (Sigma, St. Louis); and trimethoprim-sulfamethoxazole (Roche Diagnostic Systems, Istanbul).

Total chromosomal DNA was detected by the lytic effect of SDS and cetyltrimethylammonium bromide [6]. In brief, a dense suspension of bacteria was obtained from an agar plate incubated overnight and was lysed in a 1:20 volume of 10% SDS and a 1:200 volume of proteinase K for 1 hour at 37°C. The addition of a 1:6 volume of 5 $M$ NaCl and a 1:9 volume of cetyltrimethylammonium bromide and incubation for 10 minutes at 65°C completed the lysis step. DNA was extracted with chloroform/isoamyl alcohol (1:24 volume) and was precipitated with 2-propanol (0.6 volume/volume). DNA was collected by a pipette tip and was transferred to a clean Eppendorf (1.5-mL) tube; the DNA was washed 2-3 times with Tris-EDTA buffer and resuspended in 50 µL of Tris-EDTA buffer.

Random amplified polymorphic DNA (RAPD) comparison was achieved by 2 randomly selected primers and 2-step low-temperature annealing in PCR analysis. The sequences of the primers were 5'-AAC ATT TCC GTG TCG CCC TTA T-3' (authors’ unpublished data) and 5'-ATT TTC TTA GCG GCA ACT TAC-3' (OPR2) [7]. Thermal cycling was accomplished in a heated-lid thermal cycler, without overlay of mineral oil, after an initial 5-minute denaturation step at 95°C. There were 2 cycles of 95°C for 30 s,
Random amplified polymorphic DNA (RAPD) analysis (A) and restriction endonuclease (RE) analysis (B) of Shigella flexneri isolates from the central zone after the earthquake in Kocaeli, Turkey, in 1999. Lane M (A), DNA size marker. Lanes 1 and 10 (A) in RAPD (3 patterns), and lanes 7 and 8 (B) in RE comparison (2 patterns) are distinct from the rest.

Results

A total of 1468 stool cultures was processed between 20 August and 22 September 1999. According to our diagnostic procedure, 1353 (92%) of the stool samples were negative. Identified causes of diarrhea were as follows: Shigella species, 72 stools (4.9%); Salmonella species, 7 stools (0.48%); Aeromonas species, 1 stool (0.07%); G. intestinalis, 29 stools (1.98%); and Blastocystis hominis, 6 stools (0.41%).

Cases of diarrhea, relative to the size of the actual population, were scattered almost proportionally among the zones. However, analysis revealed that the cases were not distributed uniformly among the zones. Diarrheal diseases increased step-by-step after 20 August 1999 and decreased to the lowest level by 15 September 1999; the number of cases continued to be low for 1 month (data not shown). These data indicated a temporary increase in diarrheal diseases.

Discussion

Data obtained in this surveillance study overall show that diarrheal diseases increased transiently after the earthquake in Kocaeli, Turkey, in 1999. Although the etiologies of 92% of all cases of diarrhea could not be identified, the almost proportional distribution of cases between distinct zones enabled us to judge this increase as multifocal. Similarly, this study also showed that shigella infections increased in the early period (figure 1, bottom). Depending on the data obtained by phenotypic and genotypic comparisons, >1 serotype and >1 clone of Shigella caused diarrhea. Hence, given the definition of an outbreak as the dissemination of a single strain, this multiclonal increase in shigella infections was somewhat different than a...
true outbreak. Another important point is that diarrheal diseases in Kocaeli Province increased suddenly (peaking within a few days) and simultaneously in distinct zones, which is not a conventional feature of dissemination of a single microorganism from a single source.

*Shigella* is strictly a human pathogen, and dissemination of it, to some extent, reflects contamination of water and food with human feces. A multiclonal, multifocal increase in shigella infections could be due to the damaged and contaminated water supplies around the area. However, in our opinion, this multifocal increase in all kinds of diarrheal disease could not be explained only by contamination of the water supplies. This increase must be the consequence of multiple parameters, and a simple explanation could probably never be found. Beyond all other explanations, the emotional instability due to deep depression and a sudden change in living and feeding conditions of the inhabitants could also explain this multifocal increase: first, emotional instability could cause diarrhea and might be the reason for some of the cases; second, deep depression could make people less immune and more susceptible to diseases.

This study demonstrated that people are more prone to some infections after mass emergencies. Under those conditions, a surveillance system is essential to detect an increase in diseases, whereas statistical, genotypic, and phenotypic studies are essential to differentiate a true outbreak. This differentiation is especially important, because stricter measures, such as quarantining, should be applied in a true outbreak.

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**References**