Growth and Survival of Shigella flexneri in Common Bangladeshi Foods under Various Conditions of Time and Temperature

M. S. ISLAM,1* M. K. HASAN,2 AND S. I. KHAN2

Environmental Microbiology Laboratory, International Centre for Diarrhoeal Disease Research, P. O. Box 128,1 and Department of Microbiology, University of Dhaka,2 Dhaka 1000, Bangladesh

Received 10 August 1992/Accepted 18 November 1992

Survival and growth of Shigella flexneri were assessed in various foods, including boiled rice, lentil soup, milk, cooked beef, cooked fish, mashed potato, mashed brinjal, and raw cucumber. Growth at 25 and 37°C and survival at 5°C were observed by viable counts on MacConkey agar. The organism grew well in all tested foods and growth increased from 10^5 to 10^9 to 10^10 cells per ml or g within 6 to 18 h after inoculation at 25 and 37°C.

In Bangladesh, among the routine hospitalized diarrhea patients, the isolation rate of shigellae is approximately 11 to 12% (7). It causes a great deal of morbidity and mortality in Bangladesh (11, 12). In tropical developing countries, shigellosis accounts for at least 500,000 deaths per year in young children (23). Microbiologically contaminated food is responsible for a high proportion of diarrheal and other infectious diseases, particularly in developing countries. It has been estimated that the proportion of food-borne diarrheas to all diarrheas in developing countries may be as high as 70% (4).

There are many reports of outbreaks of shigellosis in which different kinds of foods are incriminated as the vehicle of transmission of Shigella spp. (1, 3, 18, 25-27). In fact, data based on public health reports indicate that food-borne Shigella infections are more common than waterborne infections in the United States and other industrialized countries (23).

In Bangladesh, women transmit the organisms through their fecally contaminated fingers while handling utensils and preparing and serving food, especially when making mashed preparations (13). However, studies of the survival and growth of Shigella species in various temperatures in various foods of Bangladesh have not been reported. Therefore, the present study was carried out to determine the role of various Bangladeshi foods as vehicles of organisms causing shigellosis.

The strain of Shigella flexneri 5 (strain 16455) used in this study was obtained from the Clinical Microbiology Laboratory of the Dhaka-based International Centre for Diarrhoeal Disease Research, Bangladesh. This strain was identified by cultural, morphological, biochemical, and serological tests, using standard procedures (10).

The strain was first inoculated onto MacConkey agar plates (Difco), and the plates were incubated at 37°C for 24 h. A loopful of the resulting growth was resuspended in 10 ml of normal saline. The transmittance of the suspension was determined at 585-nm wavelength with a spectrophotometer (Coleman Junior IIA, model 6/20). The number of cells per milliliter was assessed by viable counts, using the drop plate technique (6). Then 10^5 bacteria per ml or g was inoculated into various foods.

The kinds of food selected for this investigation depended on availability: they included cooked and raw common vegetables, fish, meat, milk, and cereals. Some of these were prepared in the laboratory, and others were obtained from the canteens of the International Centre for Diarrhoeal Disease Research, Bangladesh, and the Institute of Public Health at Mahakali, Dhaka. Boiled rice, beef, Hilsa ilisa (ilish) fish, lentil soup (dal), milk, mashed potato, mashed brinjal (the fruit of the eggplant), and cucumber were used in this study.

Prior to inoculation, all foods were autoclaved, except cucumber, and 10 g or ml of sterilized solid or liquid food was placed in presterilized petri dishes or flasks. The cucumber was peeled with a sterile knife, cut into pieces, and washed in sterile water. Then 10^5 S. flexneri cells per g or ml of food was inoculated into the flasks, and preparations were mixed by shaking and incubated at 5, 25, and 37°C. Sampling was carried out at 0-, 6-, 18-, 24-, 48-, and 72-h intervals. Ten-gram portions of solid food were blended in a blender with 90 ml of phosphate-buffered saline (PBS), and 1.0 ml of the suspension was taken from each portion; 10-fold dilutions were prepared in PBS. Each 0.1-ml portion was taken from the liquid food and 10-fold dilutions were done in PBS. Then 25 μl from each dilution was plated onto MacConkey agar, using the drop plate technique.

The plates were incubated at 37°C for 18 to 24 h, and then the number of CFU per gram or milliliter of sample was calculated.

Figure 1 shows the effect of incubation at 37°C on growth of S. flexneri in the various foods. Similar growth responses were observed in all tested foods, except rice, during the first 6 h of incubation. However, at 18 to 72 h, the highest viable count (10^10 cells per g) was recorded in mashed potato and the lowest count (10^7 cells per g) was recorded in raw cucumber.

S. flexneri showed different growth responses in different tested foods at 6 h and 25°C (Fig. 2). The best growth response was observed in cooked beef and the poorest was seen in raw cucumber. However, similar plate counts of viable cells (>10^7/g or ml) were recorded in all tested foods at 18 h of incubation. From the 18- to 72-h holding period, the maximum cell density was recorded in mashed potato and cooked fish (10^9 cells per g), and the minimum cell density was observed in raw cucumber (10^7 cells per g).

The survival of S. flexneri inoculated into various foods at
5°C is illustrated in Fig. 3. The initial inoculum level was maintained in all tested foods, except rice and milk, throughout the 72-h holding period. In rice, the initial population underwent a gradual decline in number and decreased by 1 log at 72 h. In milk, a slight reduction in the initial inoculum level was observed during the first 48 h of incubation. The cell number then returned to the initial level at the end of the 72-h holding period.

The results showed that cooked rice, lentil soup, cooked fish, and similar dishes can act as vehicles of Shigella infection. Henry et al. (5) observed that wet foods such as milk and cooked wet rice (pantha bhat), which are frequently used as infant foods in Bangladesh, contribute to the onset of acute diarrhea in children. Strains of *Vibrio cholerae* in boiled rice may reach up to 10⁵ cells per g after overnight storage (15). The present study showed that *S. flexneri* in boiled rice and milk can also undergo extensive multiplication at 25 and 37°C and a population of >10⁹/g or ml can be achieved within 6 to 18 h (Fig. 1 and 2). A similar observation was reported by Sheth et al. (22) when they examined the survival of *Shigella sonnei* in milk.

The finding that a species of *Shigella* was implicated in outbreaks of shigellosis involving cooked fish, lentil soup, and cooked beef (8) suggests that the organism was able to survive in these foods following contamination. However, the investigators did not do an in vitro study to see whether *Shigella* spp. can multiply in those foods and cause disease. The results presented here show that *S. flexneri* cells present in these foods are not only able to survive but can grow extensively at warmer temperatures and reach a population size of about 10⁸ to 10⁹/g or ml within 6 to 18 h. This is more than enough to cause infection.

The ability of *S. flexneri* to multiply in raw cucumber and mashed brinjal was also demonstrated in this study. These food items are subjected to much handling during preparation and may be susceptible to contamination. The results presented here have shown that improper storage of contaminated cucumber and mashed brinjal at high ambient temperature favors the growth of enough organisms to cause disease.

The pH of all tested foods ranged between 6.0 and 6.8 (cooked rice, 6.7; cooked beef, 6.0; cooked fish, 6.2; mashed potato, 6.0; mashed brinjal, 6.1; raw cucumber, 6.5; lentil soup, 6.4; and milk, 6.8); these values are close to the optimum for growth of shigellae. Therefore, the different growth responses of the organism in the different foods were not due to the effect of pH, but they could be nutritional. *S. flexneri* either showed a gradual decline or remained unchanged in number when refrigerated at a temperature of 5°C (Fig. 3). Refrigeration, therefore, can hardly be expected to eliminate the public health hazard in a contaminated product. The danger of shigellosis is still present for the consumer because of the low infective dose (16).

It was observed that the growth of *S. flexneri* in various foods depends considerably on the holding temperature. This finding is in agreement with a report by Taylor and Nakamura (24). A review of shigellosis in the United States revealed that the majority of the cases studied were caused by poor personal hygiene on the part of food handlers. Hand transmission is likely to be a common means of acquiring...
infection (23). Hutchinson (9) recorded that S. sonnei could survive for over 3 h on fingers. The utensils used in food preparation may also act as a source of entry into foods. Nakamura (19) showed that S. sonnei could survive on metal utensils for more than 2 to 28 days at 15°C and 0 to 13 days at 37°C. Flies may also act as carriers of Shigella species from feces to foods left uncovered (2, 14, 17, 21). S. flexneri could survive in feces for 12 days at 25°C (20). Thus, foods which were free of this infectious agent may again become contaminated. Though the level of contamination at this point may be very low, the number will increase rapidly if the conditions of time and temperature required for growth are permitted to develop.

The present study demonstrates that S. flexneri can multiply on cooked foods. In endemic areas of Bangladesh, e.g., Matlab, Teknaf, and urban Dhaka, food-borne transmission of shigellosis could be a mode of transmission within homes and in small communities where hygienic food practices and facilities for refrigerating food are lacking.

This research was supported by the International Centre for Diarrhoeal Disease Research, Bangladesh, which is supported by the aid agencies of the Governments of Australia, Bangladesh, Belgium, Canada, Denmark, France, Japan, The Netherlands, Norway, Saudi Arabia, Sweden, Switzerland, the United Kingdom, and the United States of America; international organizations, including the United Nations Development Programme, the United Nations Children’s Fund, and the World Health Organization; and private foundations, including the Ford Foundation and the Sasakawa Foundation.

We are grateful to R. B. Sack, M. Bateman, and B. A. Hoque for reviewing the manuscript, to J. Sack for editing the manuscript, and to Manzurul Haque for typing the manuscript.

REFERENCES