Shigella sonnei Isolated from Well Water

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A method is described which led to the isolation of Shigella sonnei from well water suspected of being the primary foci in a school-associated shigellosis outbreak.

In early November 1972, an epidemic of shigellosis occurred among 289 students and 25 staff members of a junior high school in Stockport, Iowa. The symptomatic disease involved 208 people.

Rectal swabs were collected by the Iowa State Department of Health and an epidemiologist from the Center for Disease Control in Atlanta, Georgia. These specimens were sent to the State Hygienic Laboratory, University of Iowa, Iowa City, Iowa. Shigella sonnei was isolated from 117 of the 263 specimens submitted.

From information obtained by the Van Buren County and the state health officials, the school water supply was implicated as the general source of infection. The school had obtained its water supply from three bored wells which were at least 38 feet deep and located approximately 10 feet from the school building. All three wells were found to be improperly covered, with two of the well casings terminating at ground level. The one operational well at the time of the outbreak was found to be improperly sealed around the outflow pipe area, and this pipe was located below ground level. The possibility of sewage contamination from the school and surrounding area could not be ruled out.

Tap water samples from a rest room of the school were collected for bacteriological examination one week after the first reported cases had occurred. These samples were found to be unsafe (coli- form most probable number [MPN] 16 + ; membrane filter total coliform 125/100 ml) by standard assay methods (1).

Based on these results, an additional large volume of water was requested for the specific purpose of isolating S. sonnei, which had been identified in the initial case by manifestation of classic symptoms.

Approximately 1,600 ml of the tap water was collected and divided into 2 samples of 800 ml. Each sample was drawn by suction through a membrane filter (Millipore Filter Co., type HA, maximum pore size of 0.45 μm). After filtration the membranes were immediately placed on xylose-lysine-deoxycholate agar (XLD) (Difco) plates (100 × 15 mm) and then were incubated at 37 C for 18 h.

After incubation the filter membrane exhibited a confluent growth of coliform organisms. A transverse sweep with a sterile loop was made across the surface of each membrane, and this inoculum was streaked to two additional XLD plates.

An average of 12 suspicious colonies from each XLD plate was picked to triple sugar-iron agar (TSI) and lysine-iron agar (LIA) slants for primary differentiation. After 18 h of incubation at 37 C, those slants of TSI-LIA exhibiting alkaline slants over acid butts without gas or H₂S were subcultured to Christensen urea slants and heart infusion broth. The urea-positive cultures were discarded after 4 h of incubation at 37 C, and the urea-negative cultures were inoculated into routine enteric differential biochemicals (4).

Of the suspect 24 colonies picked, only one was confirmed biochemically and serologically as S. sonnei. The remaining picks were slow lactose-fermenting coliforms, Proteus sp., etc.

The isolation of a causative agent from contaminated drinking water, whether it be salmonellae, including typhoid organisms, shigel- liae, etc. is rarely reported in the literature. In such epidemics, fecal contamination is usually demonstrated with the MPN coliform count and a membrane filter count, and the detection of the suspected organism is overlooked. One should bear in mind that this method of reporting should not be the sole criterion for determining fecal pollution. There are instances where the MPN of coliforms was comparatively low, or even zero, and the culture of the imputed well waters yielded Arizona and several strains of Salmonella (3, 5).

With newer products and differentiating media being marketed, techniques for the isolation of an implicated organism can be greatly
facilitated. The membrane filter technique was used by Mueller in 1947 to isolate typhoid organisms from the Hamburg public water supply during a salmonellae epidemic (2, 3). Collet and her colleagues used a similar approach in isolating Salmonella typhi from a contaminated well (3).

In these two instances salmonellae were the suspect organisms, and a bismuth sulfite broth was used for enhancement of the organism's growth as well as for inhibiting the overgrowth of other coliforms. With the use of this medium a count could be made of the salmonellae organisms when the membrane method was utilized because of the characteristic blackening of the colony (3).

It is our belief that the organism was present in very small numbers as evidenced by the isolation of only one colony from a total of 24 colonies picked. Two factors existed for the probable low number recovered: first, very few organisms were present in the initial sample specimen and, secondly, there was a 1-day delay in transporting the water to the laboratory.

It is recommended that a deliberate effort should be made in isolating the organism of a water-borne epidemic just as it is in food-borne epidemics. In most cases where water supplies are implicated in a gastrointestinal epidemic immediate-shock chlorination is applied, which destroys the laboratory's chance for recovery of any organism. Large volumes of water could be collected immediately and sent to a laboratory equipped to handle membrane filtration and eventual isolation.

With experience gained from this one epidemic, it is apparent that other media could have been included for more comprehensive isolation of the organism.

For future studies of this kind, more primary differential media will be utilized. This will involve filtering smaller samples of the water and hence more membrane filters for inoculation of prescribed media. The membranes will be placed on both selective and differential media such as eosin methylene blue, MacConkey agar, and XLD. Membranes exhibiting growth will then be placed in gram-negative broth followed by plating on the above-mentioned media, thus enhancing the chances for isolation of suspected pathogens.

This study involved one isolated outbreak of a water-borne Shigella epidemic and the eventual isolation of the suspect organism by using the membrane technique. Large-scale use of this method, with the addition of the above-mentioned modifications, may provide a helpful tool for epidemiologists in similar situations.

LITERATURE CITED