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# Indiana State Department of Health

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**DATE:** February 12, 2008  
**TO:** Thomas M. Harris M.D., Floyd County Health Department  
**FROM:** Steven Allen, Surveillance and Investigation Division  
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**SUBJECT:** Outbreak of *Escherichia coli* O157:H7 and *Shigella* in Floyd County

**Location:**  
Floyds Knobs, Indiana

**Nature of problem:**  
Fifteen *Escherichia coli* O157:H7 cases and five cases of *Shigella sonnei* were identified in a local elementary school and surrounding community.

**Date of initial contact:**  
September 20, 2007

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## Background

On September 20, 2007, the nurse at Galena Elementary School, Floyds Knobs, Indiana called the Floyd County Health Department (FCHD) nurse to report six possible *Escherichia coli* O157:H7 (*E. coli*) cases. The FCHD contacted the Indiana State Department of Health (ISDH). Floyd County is a rural area, with many houses situated on or near a farm, on the border of Louisville, KY.

## Epidemiologic Investigation

The Indiana State Department of Health (ISDH) and the FCHD immediately initiated a collaborative investigation. Case definitions established by the Centers for Disease Control and Prevention (CDC) were used to identify cases:

- Shiga toxin-producing *E. coli* (STEC)
  - Clinical description: An infection of variable severity characterized by diarrhea (often bloody) and abdominal cramps. Illness may be complicated by hemolytic uremic syndrome (HUS) or thrombotic thrombocytopenic purpura (TTP); asymptomatic infections also may occur and the organism may cause extraintestinal infections.
  - Confirmed case definition: a case with diarrhea (often bloody) and laboratory confirmation of *E. coli* O157:H7 that matched the 0710INEXH-1c cluster with enzyme patterns XbaI EXHX01.0200 and Bln EXHA26.0332.
  - Probable case definition: a case that was clinically compatible, and
    - epidemiologically linked to a confirmed or probable case, or
    - positive for shiga-toxin by enzyme immuno-assay.
- *Shigella*
  - Clinical description: an illness of variable severity characterized by diarrhea, fever, nausea, cramps, and tenesmus. Asymptomatic infections may occur.
  - Confirmed case definition: a case meeting the clinical description and *Shigella* has been isolated from a clinical specimen.
  - Probable case definition: a case meeting the clinical description that is epidemiologically linked to a confirmed case.

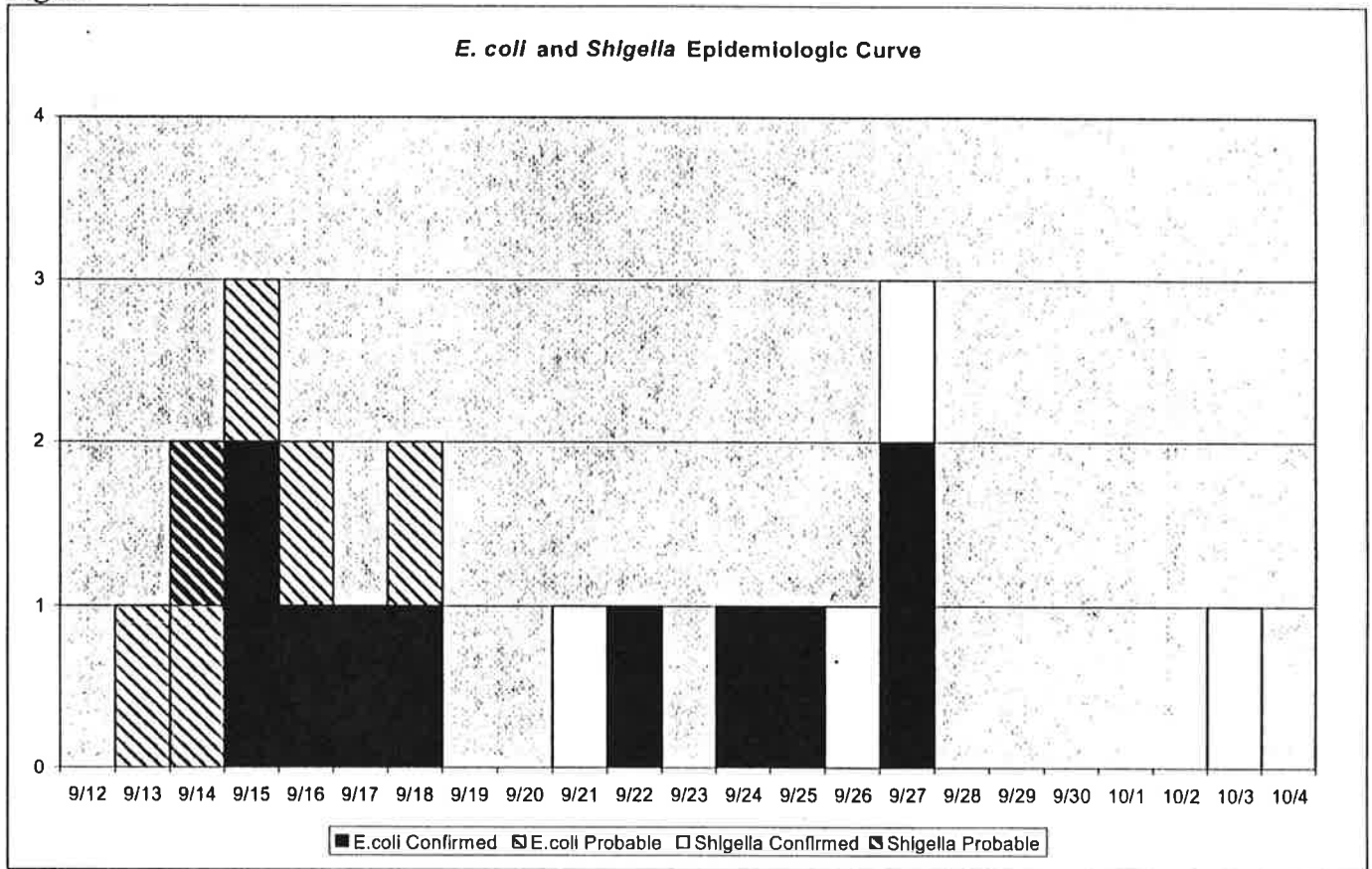
All cases were investigated using the ISDH *E. coli* and *Shigella* Case Investigation Forms to collect demographic, clinical, epidemiologic, and risk factor data. Among the cases identified early in the investigation, the only common factor was the attendance at Galena Elementary School. No restaurants or grocery stores were common among the cases. Food consumption history such as ground beef, dairy products, apple cider, raw fruits or vegetables had no association with infection. No common festivals, school field trips, family gatherings, or travel was identified. Water consumption, either well or municipal, was not a factor. Animal contact was also not a factor.

The outbreak took place during a 15 day period (see Figure 1). The majority of cases (67%) occurred between the dates of September 13 and September 18. Of the cases of both illnesses, 16 attended Galena Elementary School. The epidemiologic curve shows a pattern of transmission among the cases starting with young students at the Galena elementary school.

The investigation identified 10 confirmed cases and 5 probable cases of *E. coli* O157:H7. The *E. coli* index cases had an onset date of September 13, 2007. The FCHD was first notified about a positive *E. coli* case on September 20. Of the 15 *E. coli* cases, 53.0% were male, ages ranged from 3-39 (X = 8, mode = 6). The *E. coli* symptoms and complications included 12 cases (80%) having bloody diarrhea, 8 cases (53%) developed HUS including one adult, and 10 cases (67%) were hospitalized. All five probable *E. coli* cases developed HUS and were culture negative. It is not unusual for the *E. coli* bacteria not to grow on culture once a person has progressed to HUS as the case may have been treated with antibiotics or the bacteria are at such a low amount in the intestines, that growth is unlikely. Of the 15 *E. coli* cases, 3 were treated with antibiotics; all 3 developed HUS. This outbreak of *E. coli* was remarkable as 53% of cases developed hemolytic uremic syndrome (HUS) compared to the expected rate 8%.

The investigation identified 4 confirmed and 1 probable case of *Shigella*. The *Shigella* index case had an onset date of September 14, 2007. Of the 5 *Shigella* cases, 60% were male, ages ranged from 6-43 (X = 15, mode = 7). None of the *Shigella* cases had bloody diarrhea or were hospitalized.

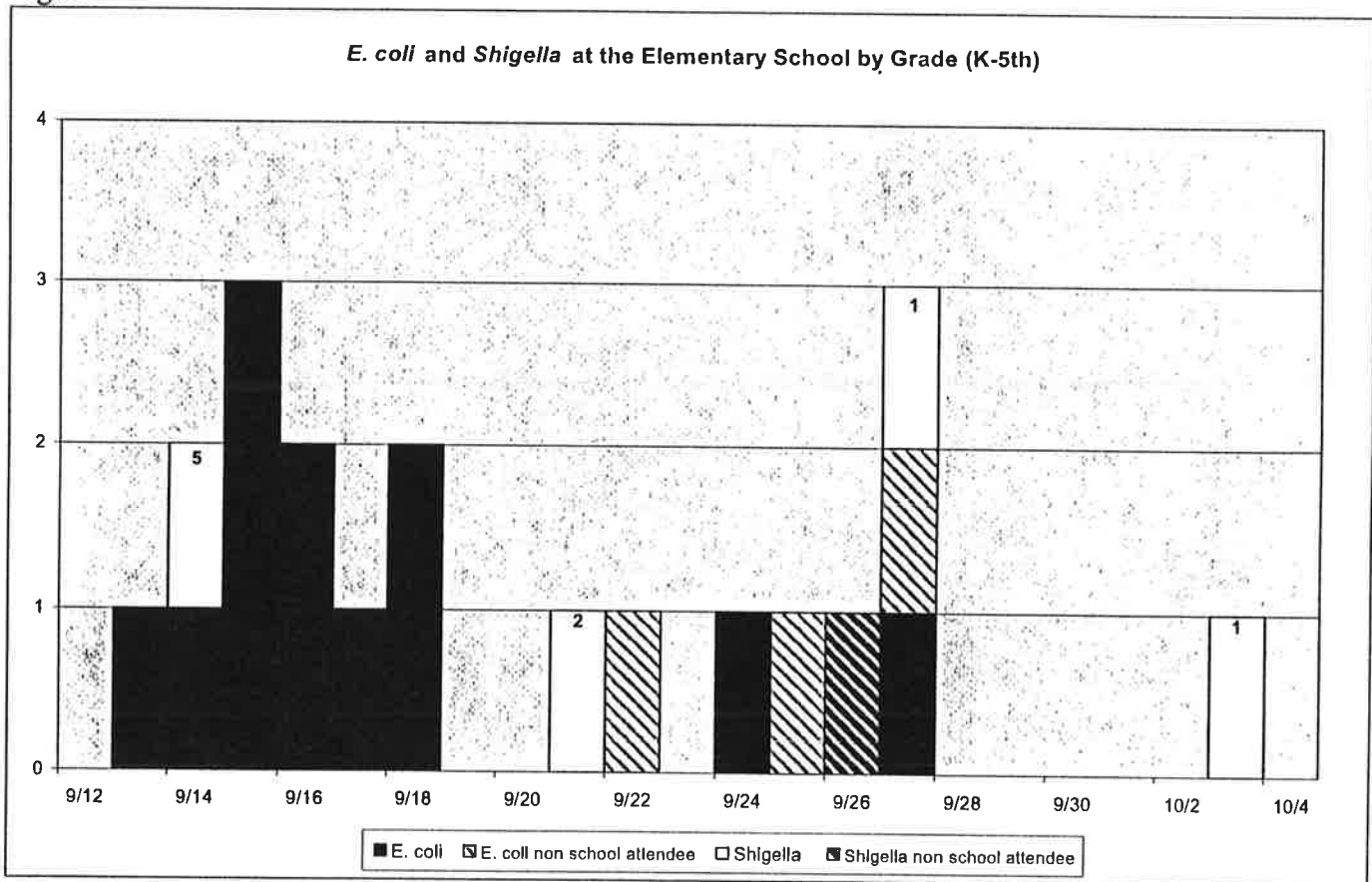
Figure 1



Galena Elementary School had 366 students attending the school at the time of the outbreak; 85.71% of the *E. coli* cases and 80.0% of the *Shigella* attended the school. The respective attack rates at the school were 3.28% and 1.13%. The ill students were from grades kindergarten (2 *E. coli*), first (8 *E. coli*; 2 *Shigella*), second (1 *E. coli*; 1 *Shigella*), fourth (1 *E. coli*), and fifth (1 *Shigella*). In all, ten different classrooms were involved. Figure 2 shows the *E. coli* cases from children who attended Galena Elementary School. The transmission of *E. coli* moved throughout the kindergarten and first grade classrooms in the first wave of the outbreak. No school employees became ill, although they would have had the same food and water exposures.

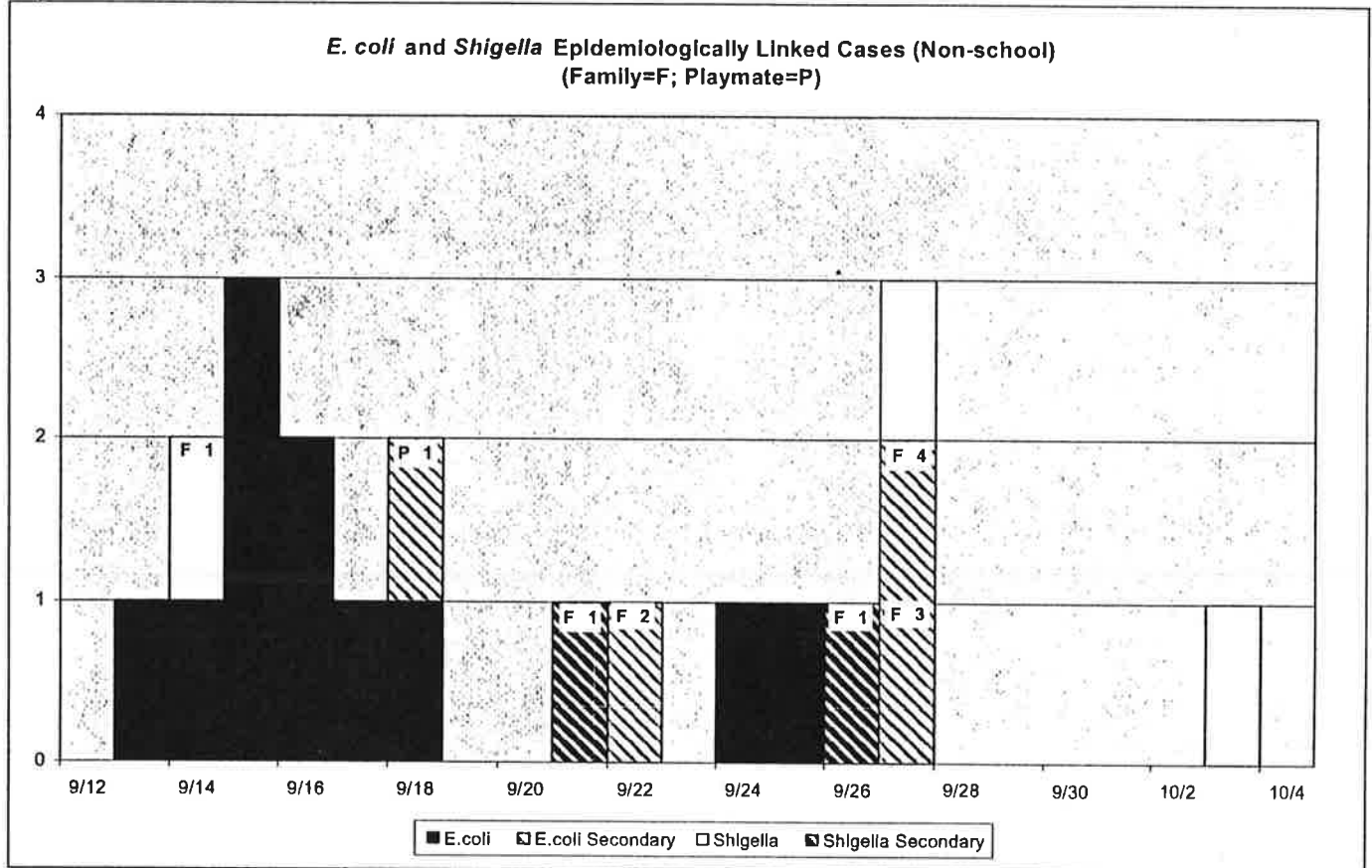
According to the Indiana Communicable Disease Reporting Rule for Physicians, Hospitals, and Laboratories 410 IAC 1-2.3, students determined to be infected with *E. coli* are able to return to school once they are asymptomatic, and students determined to be infected with *Shigella* are able to return to school once they are asymptomatic have completed at least 48 hours of appropriate antimicrobial therapy. As not all cases of *Shigella* have corresponding antimicrobial sensitivities to determine an appropriate course of action, alternatively, students are allowed to return to school after two stool specimens collected at least 24 hours apart and 48 hours after any antimicrobial therapy are negative.

Figure 2



Secondary transmission is very common among young children who are infected with *E. coli* or *Shigella*. Beyond the epidemiologic link of the school, there were links also among family members and known playmates (see Figure 3). These epidemiologic links totaled 10 cases (55%). There were three *E. coli* family clusters, one *Shigella* family cluster, and one *E. coli* playmate cluster identified. The epidemiologic curve illustrates three waves of illness, characteristic of person-to-person transmission. One family cluster (F4) of *E. coli* did not have contact with any known ill person and had indirect association with the school; other members of the family attended the school. This cluster illustrated that not all person-to-person transmission may be known and an asymptomatic infected person(s) may have been involved in the outbreak.

Figure 3



### Case Control Study

A case-control study of the *E. coli* cases was initiated on September 25 to determine similar exposures among those students that were ill and those that were not ill. The FCHD delivered the questionnaire to the hospitals and asked the nurses to interview parents as soon as possible. Seven cases questionnaires were completed and most parents were unwilling to name their child's closest friends. The case-control questionnaires focused on food preferences that were high-risk for *E. coli* dating back to September 4, a calendar of activities dating back to September 1, and lunch at the school cafeteria dating from September 4-18. None of the cases ate breakfast at the school, so this question was removed from the control questionnaire. Teachers in kindergarten, first, and second grades answered a similar questionnaire regarding their cafeteria exposures and any activities held for the students. No common risk factors were identified from the teacher questionnaires.

On September 27, the ISDH provided a control questionnaire to the school for distribution to each child in the kindergarten, first, and second grades and returned anonymously the next day. The response was overwhelming, and several parents submitted questionnaires for children in other grades. Control questionnaires were discarded if incomplete. The cases were matched to controls 1:4, first by grade, then by

gender when possible. The matched case control study ( $\alpha = 0.05$ ) produced significant results for the likelihood of food consumption from September 4 to the onset date: lettuce (OR = 15.00, p-value = 0.0221) and iceberg lettuce (OR = 34.00, p-value = 0.0047). Two food items based on likelihood were nearing significance: deli meat (OR = 9.00, p-value = 0.083) and pepperoni/salami (OR = 8.00, p-value = 0.09). No school cafeteria lunches or lunch items were significant.

On October 4, the FCHD asked parents if the investigation team could interview their ill children directly using a questionnaire based on school lunch choices from September 4-18. By that time, most cases had returned to school, so the interview was conducted at the school with the parents present. In addition to the lunch menu questions, children were asked about sharing or trading foods. Only four parents agreed to the interview. This questionnaire yielded no epidemiologic information, as two of the four children regularly brought food from home, and too few cases responded to be statistically significant.

### **Environmental Assessment**

Galena Elementary School is a single story building that is separated from the middle and high school. It has 366 students in grades kindergarten through fifth grade. There are three classes each from grades 1-5 and two kindergarten classes. Each class has approximately 20-30 students. In the week preceding the September 13 index case, 15 students were absent or sent home from school. Employees include 18 teachers, 1 principle, 1 school nurse, 3 office personnel, 3 custodians, 4 kitchen staff, 6 aids, 9 itinerate employees and 15 high school cadet teachers from Floyd Central High School. The ten children who were infected with *E. coli* rode five different bus routes.

Representatives from the FCHD and the ISDH visited Galena Elementary School numerous times throughout the investigation. On September 24, the team observed the school building layout and inspected the classrooms. All classrooms were clean and each had its own bathroom. Teachers informed the investigators that children primarily used the in-class bathrooms rather than the hallway bathrooms. All bathrooms were clean, had soap and water available for hand washing, and no water leaks were detected. Tables used during lunch periods by the students were cleaned between each use. All material safety data sheets were available, and products were acceptable for use within the school.

On September 25, the ISDH Food Protection Division visited Galena Elementary school to conduct a Hazard Analysis Critical Control Point (HACCP) inspection. Preparation processes for leaf lettuce (garnish), cut lettuce salad mix, celery sticks with peanut butter, baby carrots, watermelon, red and green grapes and creamy coleslaw were observed. All food items were prepared according to code.

The cafeteria staff used gloves and washed their hands regularly. The produce sink was cleaned after the lettuce was washed and as often as necessary. The facility used quaternary sanitizer measuring 200ppm. Three staff members were Serv-Safe trained. The ISDH Laboratory tested samples of leaf lettuce, shredded lettuce, cut lettuce salad mix, baby carrots and red and green grapes (see Laboratory Results). No violations were found during the kitchen inspection. A food production record was obtained to see what foods were served and where the food was purchased. Most foods served were prepackaged or canned and came from different suppliers. The school did not handle any raw meat.

Since lettuce was served in individual container packages, there were concerns this may have been a source of the infection. The supplier was located in Kentucky; therefore the Food and Drug Administration (FDA) and Kentucky Department of Public Health (KDPH) were informed. Subsequence inspection showed no problems, and the facility where the lettuce was prepared was exceptionally clean. Just before this outbreak, lettuce supplied by Dole Corporation was found to be contaminated with *E. coli* O157:H7. However, the lettuce used at the school was not obtained from Dole, and the strain of *E. coli* O157:H7 responsible for that outbreak was genetically different from the strain in the Floyds Knobs outbreak. In addition, epidemiological information from questionnaires ruled out lettuce as a source for *E. coli* O157:H7 at Floyds Knobs.

On September 28, the FCHD to conduct an inspection of the cafeteria during the lunch period and all practices were satisfactory. All children had cleaned their hands before eating. No children were observed sharing foods and were not allowed to do so by school staff. Kindergarten and 1<sup>st</sup> graders did not eat together. The only time the kindergarten and 1<sup>st</sup> graders had contact was on the playground.

School officials notified the FCHD that a main water pipe broke in front of the high school on September 5. The leak occurred at approximately 11:00 PM and was discovered the next day at 5:45 AM. Upon discussions with the school facilities manager and the Greenville Water utility officials on September 28, it was determined that this leak was not a suspect exposure for the illnesses. The water leak was located on a pipe that did not flow directly into either the high school or Galena Elementary School. A water valve located at each end of the broken pipe was shut off during the repair. The pressure during the leak was constant, and no backflow of water into the school buildings occurred. After repairs, two fire hydrants on each side of the fixed pipe were flushed open for approximately 30 minutes.

All school activities (i.e. Brownie meetings or field trips) during the month of September were investigated and no association was found. Any foods served at those events were either pre-packaged or came from a local pizza business. Very few of the *E. coli* cases attended those activities. One *E. coli* case attended a YMCA after-school program; this program routinely served prepackaged-snacks.

### Geographic Analysis

The ISDH used Geographic Information System (GIS) to plot the cases. Six cases were clustered around highway 150, about 1-2 miles from Galena Elementary. The other three cases resided within three miles of the school. FCHD investigated potential sources, such as a nearby small sewage treatment plant, creeks and any areas where water was found. The whole area was dry due to a severe drought in southern Indiana. It was unknown if students played in or around any water sources. FCHD staff FCHD saw nothing unusual that would warrant an investigation. On October 1, the FCHD collected two water samples from a local creek below the exit pipe from the Highlander Village Treatment Plant near the cluster of homes of ill children. The water samples were sent to the ISDH Laboratory for analysis (see Laboratory Results).

### **Laboratory Results**

The KDPH Laboratory and the ISDH Laboratory conducted confirmatory testing and pulse-field gel electrophoresis (PFGE). All 10 confirmed *E. coli* cases had identical PFGE patterns. The Indiana outbreak had a cluster name of 0710INEXH-1c with enzyme patterns XbaI EXHX01.0200 and Bln EXHA26.0332. The national PulseNet database confirmed that this *E. coli* pattern was not reported anywhere else in the nation during the previous 90-day period.

Food samples tested were negative for *E. coli* O157:H7. Three samples showed elevated aerobic plate counts indicating the potential for temperature abuse or mishandling (see Table 1).

Table 1: Food results collected from the Galena Elementary Cafeteria

Sample	APC Count (cfu/g)	<i>E. coli</i>
Shredded lettuce	2.0 x 10 <sup>6</sup>	negative
Leaf lettuce	3.0 x 10 <sup>5</sup>	negative
Cut salad mix	7.3 x 10 <sup>6</sup>	negative
Baby carrots	-	negative
Green grapes	-	negative
Red grapes	-	negative

The ISDH Laboratories conducted testing on water samples collected from the creek. Sample 835-1 tested positive for coliform (190 cfu/100 ml) with presence of *E. coli* bacteria and Sample 835-2 tested positive for coliform (579 cfu/100 ml) with the presence of *E. coli* bacteria. Acceptable levels for surface water and swimming/bathing beaches should not exceed 235 cfu/100 ml. These results illustrated that surface water in the environment can be contaminated above acceptable levels for safe swimming/bathing. The *E. coli* samples from the creek were determined not to be a shiga-toxin producing species or *E. coli* O157:H7.

### **Conclusions**

The investigation indicated that the outbreak of *E. coli* O157:H7 and *Shigella* did not originate from a Galena Elementary School meal or event and rather was likely the result of person-to-person transmission. Due to the discriminate nature of the *E. coli* and *Shigella* illnesses in certain ages, grades, and classrooms, waterborne transmission would be highly unlikely, since illnesses would be expected in all ages throughout



the community. This outbreak of *E. coli* was remarkable as 53% of cases developed hemolytic uremic syndrome (HUS) compared to the expected rate 5-10% but can be higher during outbreaks. Of note, three of the HUS cases were treated with antibiotics which may have exacerbated their *E. coli* infection. According to The Red Book, *E. coli* infections should not be treated with antibiotics as no benefit has been proven, and some studies have suggested that children infected with shiga-toxin producing *E. coli* have a greater risk of developing HUS when compared with children not treated with antimicrobial agents.

Case control study had limited value. Due to the severe illness this particular *E. coli* O157:H7 strain, it was difficult to get many of the questions asked from the ISDH investigation form. Many parents were distraught or busy taking care of their children in the hospital. Another difficulty was parents trying to remember the foods their children ate. Children are difficult to interview as their understanding of past events and their memory would likely hinder the investigation results. The case control study had a small sample size (n=7) and was unlikely to capture data needed for a quality study. While the case control study suggested that an ill case was 15 times more likely to eat lettuce than a non-ill person from Galena Elementary during September 4-18, this data was based on a likelihood of exposure. However, the case questionnaires indicated that only one ill student ate salad for lunch at the school on one day. All lettuce provided to the school was not included in the Dole Lettuce recall, and no violations were identified at the preparation facility.

Since no environmental or food exposure was identified throughout the investigation, it is likely that the outbreak originated from person-to-person transmission within the school. Most cases were 6 – 8 years old and were considered less likely to practice the personal hygiene needed to prevent the spread of illness. Also, the identification of *Shigella*, another bacterial disease highly associated with person-to-person transmission, further demonstrates this route of direct transmission among the students. The investigation identified 16 children that attended the school, of which 12 students had close contact with another student in the same grade infected with *E. coli* or *Shigella*. Also, the investigation identified five epidemiologically linked clusters (n=11) illustrating person-to-person transmission in the home or among playmates. Both *E. coli* and *Shigella* can be shed in the stool while the infected person is asymptomatic or after the person has recovered from illness. This outbreaks showed the importance of personal hand hygiene in schools, the need for removing ill children with diarrhea from school, the need for children with diarrhea or recovering from diarrhea to see a health care provider, and those ill children to have minimal contact with others until fully recovered.

Symptoms of *E. coli* infection, including bloody or non-bloody diarrhea, abdominal cramps usually begin 3-4 days (range of 2-10 days) after exposure and last for approximately 5-10 days. Some people may only have mild diarrhea without blood or no symptoms at all. An infected person can pass the bacteria in their stool for up to 3 weeks after their symptoms have stopped. People infected with *E. coli* (O157 and other shiga-toxin producing strains) can develop a condition called hemolytic uremic syndrome (HUS). This condition is very serious and can lead to kidney failure and death. Children under 5 years of age and the elderly are more likely to develop this condition.

Symptoms of shigellosis, including stomach cramps, diarrhea, fever, vomiting, and blood, pus, and mucus in the stool, usually begin 24-72 hours (range of 12 hours to 5 days) after exposure and last about 4-7 days. Some people may have no symptoms but can still spread the infection to others.

In general, *E. coli* and *Shigella* infection can be prevented by strictly adhering to the following guidelines:

- Practice good hygiene:
  - Thoroughly wash hands with soap and water after using the restroom; after assisting someone with diarrhea and/or vomiting; after contact with animals and reptiles; after swimming; before, during, and after food preparation; and after exposure to raw meat products (please refer to the [ISDH Quick Facts about Hand Washing](#)).
  - Clean food preparation work surfaces, equipment, and utensils with soap and water before, during, and after food preparation, especially after contamination with raw meat products.
- Protect others:
  - Persons with diarrhea and/or vomiting should not prepare food or provide health care for others and should limit direct contact with others as much as possible.
  - Persons with diarrhea and/or vomiting should not attend a day care facility or school.

- Persons with diarrhea and/or vomiting shall be excluded from employment involving food handling (Indiana Retail Food Establishment Sanitation Requirements, 410 IAC 7-24-122).

In addition, *E. coli* infection can be prevented by strictly adhering to the following guidelines:

- Separate raw and cooked foods:
  - Avoid cross-contamination by keeping uncooked meat products separate from produce, ready-to-eat foods, and cooked foods.
  - Use separate equipment and utensils for handling raw foods, especially for marinades or barbeque sauce.
  - Clean food-preparation work surfaces and utensils with soap and water before, during, and after food preparation, especially after contact with raw meat products.
- Maintain safe food temperatures:
  - Ensure proper temperatures are maintained during refrigeration (<41°F), freezing (<0°F), holding (keep food hot or at room temperature for no longer than 4 hours), and chilling (chill immediately and separate into smaller containers if needed).
  - Thoroughly cook all food items to USDA recommended safe minimum internal temperatures:
    - 145°F – steaks and roasts
    - 160°F – pork and ground beef (should not be eaten pink)
- Eat safe foods (Remember: Contaminated foods may look and smell normal):
  - Do not eat undercooked meat.
  - Do not eat foods past the expiration date.
  - Do not eat unpasteurized dairy products and fruit juices, including apple cider; it is illegal to sell unpasteurized dairy products in Indiana.
  - Wash all produce before eating raw or cooking.
  - Use treated water for washing, cooking, and drinking.
- Handle animals safely:
  - Wash hands after contact with livestock, petting zoos, pets, especially if they are suffering from diarrhea.

## Recognition

The ISDH recognizes the work Floyd County Health Department conducted during this outbreak investigation. Many staff members worked long and hard to investigate these illnesses. The ISDH also recognizes the Galena Elementary School staff and school superintendent's office staff for their full cooperation. The ISDH acknowledges their extra efforts to accommodate the FCHD and the ISDH personnel and allowing them unlimited access to the school. Staff members at Galena Elementary School were very caring and helpful during the investigation. The ISDH also appreciates the collaboration with the Kentucky Department of Public Health in the investigation. The ISDH thanks the laboratory, infectious control, and nursing personnel from Floyd Memorial Hospital, Clark Memorial Hospital, Norton Healthcare (Kentucky), Kosair Children's Hospital (Kentucky), Harrison County Hospital, and the Kentucky Department of Public Health (KDPH) for their efforts in collecting, analyzing, and transporting specimens quickly. They were very accommodating when asked to fax laboratory results, clinical reports, and updated patient information to the FCHD. This was a classic example of public health professionals working together with other partners to prevent the spread of disease. Thanks again to all who helped.

## References

1. Communicable Disease Reporting Rule for Physicians, Hospitals, and Laboratories. 410 IAC 1-2.3. October 11, 2000. Pages 27-28.
2. Red Book. 7<sup>th</sup> Edition. 2006. American Academy of Pediatrics. Pages 291-295.