

# A Medical Mystery of Epidemic Proportions

## Daphne's Blog - Sunday

I'm not sure my decision to be a Peace Corp volunteer was a good idea. I thought I was prepared for working in a village where extreme poverty and lack of transportation made obtaining food, water and medical care difficult. But, this has become really scary!

When I look out of my tent, I can see sick villagers curled up in the shade of a tree. They are waiting to be admitted to an already crowded hospital tent. One girl, weak and dehydrated, died of the severe diarrheal disease before she could get treatment.

We had a very heavy rainstorm here on Friday. I think that the rain's runoff carried human waste into the village's water supply. It's like the villagers are drinking poison. I'm afraid that this might be the beginning of a cholera epidemic. Here's what I found on the Internet about cholera.

**Cholera** is an intestinal infection caused by ingestion of food or water contaminated with the bacterium *Vibrio cholerae*. It has a short incubation period, from less than one day to five days.

*Vibrio cholerae* bacteria produce a potent toxin that causes a large amount of watery diarrhea that can quickly lead to severe dehydration and death if treatment is not promptly given.

Cholera is usually transmitted through water or food that is contaminated by human feces containing *Vibrio cholerae* bacteria. Cholera outbreaks can occur in any part of the world where water supply, sanitation, food safety, and hygiene are inadequate.

1. What bacterial pathogen causes cholera?

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2. How do these bacteria lead to the death of people who are not given prompt treatment?

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3. How is cholera spread from one person to another?

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## Part I: What is causing this disease outbreak?

### Daphne's Blog - Monday

An emergency medical team arrived and set up a hospital tent. They brought boxes of IV bags and antibiotics provided by American donors. Prompt treatment of diarrheal disease with an IV fluid is critical! The patients can die very quickly if IV's aren't started immediately to replace the fluids that victims have lost.

This epidemic is horrible! There are many new patients today. The hospital beds are just cots with a hubcap-sized hole cut in the middle. A bucket sits under the hole. Another bucket rests on the floor next to the victim's head. The patients are too weak to speak. Groans, sounds of vomit and diarrhea splashing into the buckets, and a horrible stench fill the crowded hospital. The hospital tent's 110 beds are full but the patients keep arriving.

There are many pathogens that could cause an outbreak of diarrheal disease. Luckily they have a *Vibrio cholerae* Rapid-Test Kit that can determine if this outbreak is caused by *Vibrio cholerae* bacteria. If this is a cholera outbreak, it will be important to take quick action to prevent the spread of *Vibrio cholerae* bacteria to other villages.

1. Use the *Vibrio cholerae* Rapid-Test Kit to test a sample of well water from the village and a diarrhea sample collected from a patient.
2. Record your laboratory observations by coloring or labeling the pictures of the dipsticks.
3. Record your conclusions based on the Rapid-Test results. Does the test show that *Vibrio cholerae* are present?

Well Water Sample	Diarrhea Sample	<b><i>Vibrio cholerae</i> Rapid-Test Kit</b>
		<b>Laboratory Observations</b>
		<b>Conclusions:</b>
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		_____
		_____
		_____

## Part 2: Why are people who should be immune getting sick?

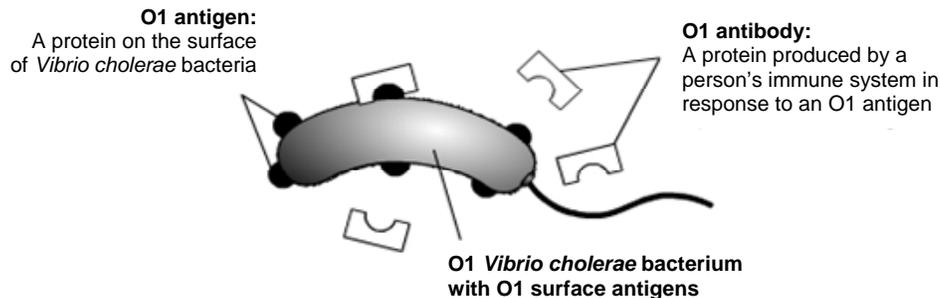
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### Daphne's Blog - Wednesday

Two members of the medical team have developed cholera. That shouldn't be happening because they both had cholera vaccinations. People who had cholera before are also getting sick. This is turning into a very scary medical mystery! Why are people who were vaccinated or who had cholera before getting sick?

According to the doctors, only one type of *Vibrio cholerae* bacteria causes epidemic cholera – this is known as the O1 type. People who have been infected with cholera in the past and people who had cholera vaccinations should be immune to the O1 type of cholera. Their immune system should be making O1 antibodies that bind with and destroy the O1 type of *Vibrio cholerae*.

**Type O1 *Vibrio cholerae* have O1 antigens on their surface. When a person is exposed to O1 *Vibrio cholerae*, their immune system produces specific O1 antibodies. These antibodies bind to O1 antigens and destroy the O1 *Vibrio cholerae*.**



The emergency medical team is concerned that a new type of *Vibrio cholerae* bacteria might be causing this outbreak. If that's true, then the new type of *Vibrio cholerae* may not have O1 antigens. That would mean that the O1 antibodies cannot bind to and destroy the new type of *Vibrio cholerae*.

To determine if this is an O1 type or a new type of *Vibrio cholerae*, you can do an antibody test that uses O1 antibodies. If the O1 *Vibrio cholerae* bacteria are present in the patient sample, the O1 antibodies should bind with the O1 antigens on the bacteria surface. The binding will cause the *Vibrio cholerae* to clump together.

1. Which type of *Vibrio cholerae* bacteria is known to cause cholera epidemics?

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2. What is the difference between an O1 antigen and an O1 antibody?

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3. Explain why people who were infected by O1 *Vibrio cholerae* before should not get sick when they are exposed to this pathogen again.

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4. What results should you observe if O1 antibodies are mixed with O1 *Vibrio cholerae* bacteria?

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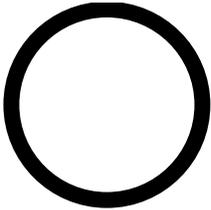
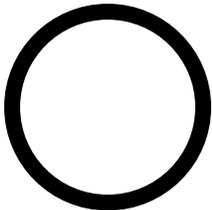
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5. Use the Antibody Test Kit to test the *Vibrio cholerae* sample from this cholera outbreak.

6. Record your laboratory observations indicating whether the samples clumped or did not clump.

7. Record your conclusions based on the results of the antibody testing.

**Antibody Testing Laboratory Observations**

<p>O1 Antibody solution</p>  <p>Known O1 <i>Vibrio cholerae</i> (Positive Control)</p>	<p>O1 Antibody solution</p>  <p><i>Vibrio cholerae</i> sample from patient</p>
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**Conclusions:**

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8. Why was it important to test a known sample of O1 *Vibrio cholerae* when using the antibody test kit?

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9. Explain why the adults who had cholera in the past or who had been vaccinated for O1 type *Vibrio cholerae* are not immune during this cholera epidemic.

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## Part 3: How did O139 evolve?

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### Daphne's Blog - Friday

OK, one mystery has been solved. This cholera outbreak is caused by a **new** type of *Vibrio cholerae* called **O139**.

But now there is a new mystery. How did this new O139 *Vibrio cholerae* evolve? The scientists I've been talking to have two hypotheses.

**Hypothesis 1:** The new O139 *Vibrio cholerae* evolved from a non-pathogenic (harmless) type of *Vibrio cholerae* that picked up a gene for the cholera toxin. If that happened the harmless type could become a "killer" *Vibrio cholerae* that is not recognized by O1 antibodies.

**Hypothesis 2:** The new O139 *Vibrio cholerae* evolved from an O1 *Vibrio cholerae* that mutated and lost the ability to make the O1 antigen. If that happened, people's O1 antibodies also wouldn't be able to recognize the new O139 *Vibrio cholerae*.

To see which hypothesis is correct, the doctors have sent a sample of the new O139 *Vibrio cholerae* to the CDC (Centers for Disease Control). Researchers at the CDC will use a DNA test called a "microarray" to compare the genes of the O1 *Vibrio cholerae* with the genes of the new O139 *Vibrio cholerae*.

1. Observe diagrams A and B on the colored graphic sheet in your lab kit.

- Which diagram best illustrates Hypothesis 1? \_\_\_\_\_

Explain your answer:

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- Which diagram best illustrates Hypothesis 2? \_\_\_\_\_

Explain your answer:

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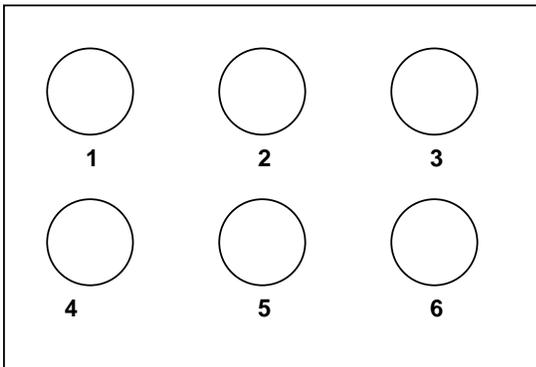
2. Your lab kit contains a simulated DNA microarray has been spotted with DNA from many different O1 *Vibrio cholerae* genes. Your kit also contains a tube of simulated DNA isolated from the O139 *Vibrio cholerae*.

3. Follow the instructions in the DNA Microarray Test Kit to test the O139 DNA sample.

4. Record your laboratory observations below by coloring in the DNA spots that are pink.

5. Record your conclusions from the microarray testing.

### DNA Microarray Laboratory Observations



#### O1 *Vibrio cholerae* genes spotted on the microarray:

- Gene 1 – Cholera toxin gene
- Gene 2 – Catalase enzyme gene
- Gene 3 – DNA polymerase gene
- Gene 4 – Flagella gene
- Gene 5 – O1 Antigen gene
- Gene 6 – Pilus gene (attachment to intestine)

Conclusions:

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6. Which hypothesis is supported by the results of your DNA microarray analysis?

- Hypothesis 1: O139 evolved from a harmless *Vibrio cholerae* that picked up a gene for the cholera toxin.
- Hypothesis 2: O139 evolved from an O1 *Vibrio cholerae* that lost the gene for the O1 surface antigen.

Hypothesis: \_\_\_\_\_

Support your answer with evidence from the DNA microarray testing.

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7. Why is the evolution of a new type of *Vibrio cholerae* a serious health threat?

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## Part 4: What can be done to prevent cholera epidemics?

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### Daphne's Blog - Sunday

The medical team says that we need to take action to prevent additional cases of cholera. They've asked me to work with people who live in this village and nearby villages to make them aware of what they could do to prevent the spread of the O139 *Vibrio cholerae*.

The immediate solutions seem so simple. Encourage villagers to use the temporary supply of clean water provided by the medical team. Drill wells that would provide clean water. Build latrines so that villagers wouldn't use their fields as open-air toilets. But these options are too expensive unless the government steps in.

Hopefully, in the future, scientists will develop a new vaccine that protects people from the O139 *Vibrio cholerae*.

1. What actions should villagers take to prevent O139 type *Vibrio cholerae* from causing future cholera epidemics?

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2. Why are implementing these actions difficult in many areas of the world?

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