Reinforcing the Importance of Hypotheses in the Scientific Method of Inquiry:
A Learning Activity Using the 2006 Spinach Contamination Event

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Introduction

The primary goal of this learning activity is to help students understand the importance of the critical role of hypotheses in the scientific method of inquiry, the orderly means of investigation to answer questions in scientific situations. A second goal is to link this concept to the bacterial contamination of food. Not only does this invoke enthusiastic interest in an unseen, perhaps neglected domain of life, but it additionally fosters realism, as students' futures may involve contamination dilemmas. They are the next generation of physicians, researchers, communicators and public policy makers who need the knowledge background to deal effectively with issues such as nutrition, health, safety, and wellness.

To achieve these goals, we engage students in studying the September 2006 event of spinach contamination by *Escherichia coli* via hands-on and minds-on activities, combined with opportunities for group research and discussion. It has been reported that the leafy green vegetables are a common source of *E. coli* infections in the United States, second only to ground beef. By studying this particular incident of bacterial contamination of spinach, students can become directly involved in the process of the scientific method of inquiry, using a real world example. After all, science, which means to know through the exercise of reason, is a construction of the mind based on actual observations to find better explanations to the natural phenomena of the world around us. Science, by choice, "is limited to questions that can be approached by the use of reason, questions that can be answered by the discovery of objective knowledge, and the elucidation of natural laws of causation" (Futuyma, 1983, p. 170). The practice of the discovery of objective knowledge involves observation of events (or the acquisition of data), followed by inference of the possible causes of the events (forming alternative hypotheses), and finally, testing the inferred causes (to reject insufficient hypotheses, and select the best explanation).

Spinach and *Escherichia coli*

Spinach (*Spinacia oleracea*) belongs to the family Chenopodiaceae and is a popular leafy green vegetable. Thanks to genetic engineering, as well as to green house farming, fresh spinach is grown under all types of environmental conditions and is available in the United States year round. Its best growing seasons, however, are from March to May, and again from September to October. According to the United States Department of Agriculture, Americans consume about 450 million pounds of fresh spinach every year. The vast majority of this, about 75%, comes from California.

Spinach is an excellent source of a number of essential nutrients including vitamin C and vitamin A-rich beta carotene. Together, they work to reduce the amounts of free radicals in the body---vitamin C as a water-soluble antioxidant, and beta-carotene as a fat-soluble one. Some researchers have suggested that eating spinach could help protect against osteoporosis, heart disease, colon and prostate cancer, arthritis, and other diseases (Murray 2005, Pratt and Mathews 2005, Margen 1992). Furthermore, researchers have identified at least 13 different flavonoid compounds in spinach that function as antioxidants and as anti-cancer agents. For example, a carotenoid found in spinach and other green leafy vegetables is useful in fighting human prostate cancer in two ways. First, it induces prostate cancer cells to self-destruct. Second, it "is converted in the intestines into additional compounds, called neochromes, which put prostate cancer cells into a state of stasis, thus preventing their replication" (Mateljan, 2007).

However, spinach's nutritional worth was made secondary to concerns of its consumable safety on September 14, 2006, when the U.S. Food and Drug Administration (FDA) recalled all fresh spinach from supermarket shelves. The recall was announced after severe illness was reported among people who had consumed the produce. According to the FDA, more than 170 people in 25 states had become sick, and at least two deaths had been attributed to the eating of the fresh spinach contaminated with a particular strain of *E. coli* called O157:H7. This strain of *E. coli* not only

causes the cramps and bloody diarrhea typically associated with bacterial infections, but can also lead to serious complications such as hemolytic uremic syndrome (HUS). In HUS, *E. coli* O157:H7 in the infected person's intestine produce a toxin that then enters the blood stream and starts to destroy red blood cells. This syndrome, which affects children more than adults, can lead to serious complications including kidney damage and acute kidney failure. Twenty-seven cases of HUS were reported as a result of the fall 2006 spinach contamination event.

The FDA lifted the ban on the sale of fresh spinach on October 2, 2006. The agency announced that the fresh spinach on supermarket shelves was safe to consume, and that tainted spinach was no longer being sold. Consumers were advised to avoid any leftover, possibly contaminated bags, and to not purchase any fresh spinach with a 'use by' date of October 1, 2006 or earlier. In the end, more than one company had been implicated in the outbreak of *E. coli* O157:H7 bacteria in bagged spinach. Throughout the "*E. coli* contaminated spinach episode" in September 2006, many people were afraid that the contaminated fresh spinach might be a bigger problem and of a greater geographical distribution than what was reported.

Many people perceived bacteria as the 'villain,' the 'bad guy,' totally uninformed of the important role of microorganisms on the planet, and their part in the ecosystem of life. As Koneman suggests, "whether their actions are damaging or fatal, or just bad manners, they merely play out what is encoded in their chromosomes. ... but even they simply act as they are created to act, their sole purpose being to survive and to replicate" (2002, p. 9). It is not the bacteria to blame for the contaminated spinach, but those who made it possible for the *E. coli* to be introduced into spinach fields and use spinach as a host to proliferate and produce progeny. Additionally, enough attention may not have been focused on how to effectively kill the bacteria present in the spinach; while washing alone is not able to kill the bacteria, cooking the spinach properly can. After all, *Escherichia coli* which is one of the most widely studied of prokaryotes, is "most versatile in its extent of animal and human colonization, and most innovative in its varied expression of virulence mechanism" (Koneman, 2002, p. 11). Remarkably, they swap drug-resistance genes to survive, and they use living organisms, including humans, as platforms for carrying them around.

Minds-On Activity and Discussion

Reinforcing the importance of hypotheses in the scientific method through the study of bacteria and bacterial contamination

Dr. Lilly Johnson, microbiologist, decided to give her students an assignment to find out whether or not *E. coli* love spinach. Dr. Johnson's students were very excited to deal with current issues, and to engage in a study that might make a significant contribution to society. As Colburn (2008, p.12) has argued, "The more concrete and familiar materials and ideas in an activity are to students, the more successful they will be in figuring things out for themselves."

Dr. Johnson divided her class into groups of four students each. She asked each group to formulate a number of hypotheses, then to eliminate them one by one using logic until they were left with one hypothesis that could not be eliminated. Each group would then perform experiments to empirically test the lone remaining hypothesis. Dr. Johnson asked that each group get her approval for the final selected hypotheses before beginning their experiments.

Dr. Johnson received the final hypothesis from each group. She examined all the proposed hypotheses and rejected all of them without giving her students the reason why. She asked them to go back and try again. Table 1 contains the final hypotheses receive from the 6 groups and her decision for each one of them.

Table 1: Hypotheses & Decisions

Group	Final Hypothesis	Dr. Johnson's Decision
1	Bacteria multiply faster by binary fission when grown in medium containing spinach.	**REJECTED**
2	All bacteria isolated from fresh spinach will cause disease in humans.	**REJECTED**
3	Spinach has no effect on the rate of binary fission of bacteria when bacteria are grown in medium containing spinach.	**REJECTED**
4	Spinach increases the toxicity of bacteria when bacteria are grown in medium containing spinach.	**REJECTED**
5	Bacteria that can be isolated from fresh spinach, but not from fresh collard greens, are the bacteria that caused people who ate spinach to get sick.	**REJECTED**
6	Bacteria multiply slower than normal by binary fission when grow in medium containing spinach.	**REJECTED**

To achieve the goals of this learning activity, students need to be engaged in formulating answers (after careful small-group or whole class discussion) of the following questions.

1. On which basis, after conducting a given experiment, do scientists discard, revise, or accept a given hypothesis?
2. Why did Dr. Johnson reject all the proposed hypotheses?
3. Do you agree with Dr. Johnson's decision regarding each hypothesis? Explain.
4. How can you improve the students' hypotheses by rewriting each, so they can be resubmitted for approval by Dr. Johnson?
5. Transfer each rewritten hypothesis into investigative questions that can be answered empirically.
6. What are the differences that distinguish (in science) hypotheses from theories?
7. Discuss how an observation may lead to generation of a hypothesis, and how experimentation may follow.

Related Research & Discussion Assignments

For the activities that follow, we suggest that classes be broken into small groups, with each group being assigned one or two of the research/discussion assignments, and then reporting back to the whole class on the outcomes of their work. To achieve the goals of this learning activity, students need to be engaged in research from a variety of sources, including relevant journals and books, along with credible Internet sources to answer the following questions. These questions are aligned with the topics of bacteria, bacterial contamination and the scientific process.

Research & Discussion (I)

Discussion of the questions following individual background research is recommended to ensure the most logical answers.

1. What do you know about the Domain Bacteria?
2. Looking specifically at the September 2006 *E. coli* incident, answer the following:
   a. What was the cause or the source of the September 2006 nationwide outbreak of *E. coli* contaminated spinach?
   b. What evidence was reported that matches the bacteria strain which killed three Americans and sickened 200 others to the *E. coli* from the proposed source?
   c. From your own perspective and from your research findings, was the evidence conclusive enough to link the bacteria strain which killed three Americans and sickened 200 others to the *E. coli* from the proposed source?

d. According to your research, was the proposed source of *E. coli* physically close (or in some other way connected) to the environment where the contaminated spinach or lettuce was grown in September 2006?
e. Why were the spinach farmers in the state of California more worried than farmers in the other states?

3. California farmers (who grow three-quarters of the nation's spinach) suggest that it is an impossibility to grow these vegetables outside, with a solid guarantee for a safe product, without drastic measures being introduced--- as it would be if all of the Salinas Valley was turned into a virtual 'greenhouse.' Why do you think the spinach growers are concerned that this approach might reduce the sale of spinach in the market, and in turn reduce the consumption of the product?
4. According to your Internet research, what other sources have scientists examined as a source of the September 2006 *E. coli* O157:H7 strain outbreaks in spinach?
5. Is spinach associated with any common allergic reactions?
6. Does the testing of spinach for bacterial contamination differ from one growing season to another according your research?
7. Are there any safety concerns about eating spinach?
8. Where is spinach thought to have originated?
9. How did spinach make its way to Europe?
10. Which country and/or region in the world is considered to be the largest commercial producer of spinach?
11. How many different types of spinach does your research reveal?

Research & Discussion (II)

Discussion of these topics can result in the most logical answers. The three following activities are repeated (with permission) from Cherif, Michel, Movahedzadeh, Adams, and Aron (August 2009).

1. Conduct Internet research to find out why Fred Pritzker and Rich Ruohonen were called '*E. coli* Lawyers.' In addition, they both were selected by other lawyers for inclusion in *The Best Lawyers in America.*
2. Conduct Internet research to find out:
   - How *E. coli* infections are caused.
   - What the symptoms of *E. coli* foodborne illness are, including initial symptoms, Hemolytic Uremic Syndrome (HUS), and Thrombotic Thrombocytopenic Purpura (TTP).
   - How *E.coli* infections are diagnosed and treated.
   - How *E. coli* infections can be prevented.
3. Anaphylaxis is a severe, sudden, often fatal, whole-body allergic reaction to a foreign substance or antigen that leads to the person's immune system becoming sensitized to that allergen. As a result, the body's tissues release histamine and other substances, with possible tightening of the airways, and other concomitant symptoms. This is a rare event in humans, usually triggered by antiserums (such as to treat snake or insect bites), antibiotics (immunoglobulin production especially), or chemical agents (introduced via bee or wasp stings). As well, certain foods can initiate these severe reactions, as evidenced with allergic reactions to seafood (shellfish especially), rice, potatoes, peanuts, egg whites, raw milk, and pinto beans (Wardlaw and Smith 2009, Gotto 2002, Komaroff 1999). Conduct Internet research to find out:
   - The symptoms of anaphylaxis and anaphylactoid reactions.
   - Whether spinach causes anaphylaxis.
   - Whether *E. coli* causes anaphylaxis.

Research & Discussion (III): Keep Bacteria Away

Not all bacteria are harmful; indeed, the vast majority of bacteria are harmless. What is more, many of them are helpful for humans, and some others are even *essential* for human life. As well, though spinach may harbor bacteria--- good and bad--- on its own, it is a tremendous source of nutrients. Conduct Internet research to:

1. Identify the main differences between bacteria that cause disease, and bacteria that do not cause disease.
2. Find out the many divergent ways to minimize exposure to possible harmful bacteria that could lead to food borne illnesses. Note use of extremes such as hot versus cold exposure, high versus low pressure situations, and cleanliness as possible guidelines. Then, following thorough discussion, answer the questions below and complete the data table with the required information. Resources that can be used include NIH

Publication No. 07–4730 - May 2007, Bacteria and Foodborne Illness
http://digestive.niddk.nih.gov/ddiseases/pubs/bacteria/

- What is the current protocol for washing of hands before and after handling of food?
- In regards to facial and body hair, what is the current protocol if one works directly with food preparation? What regulations are in place if one serves food?
- How should one handle meat, poultry and seafood juices in food preparation? What method should be used to clean up of these juices on particular surfaces?
- What is the protocol for storage of raw meats, seafood, and produce?
- How long are refrigerated foods allowed to be out of refrigeration in common situations? Is there a difference in these allowed times if the food is mayonnaise based?
- Cooking food is one of the mechanisms of preventing foodborne illnesses in society. Fill in Table 2 with the correct internal temperature needed to cook the item thoroughly to discourage foodborne illnesses.

Table 2: Internal Temperatures Necessary for Thorough Cooking

Food Source	Internal Temperature (°C/ °F)
Pork (1 lb) boneless	
Pork (1 lb) with bone	
Chicken (1 lb) boneless	
Chicken (1 lb) with bone	
Beef (1 lb) boneless	
Beef (1 lb) with bone	
Shellfish (1 lb)	
Fish without shell (1 lb)	

3. As a possible follow up exercise, have students research restaurants and grocery stores in their area. Have them make note of signage for the proper washing of hands by those preparing or serving food in locations such as kitchens and washrooms. Also, look up regulations authorized by the Center for Disease Control (CDC) and other agencies involved in safe food handling and the food service industry.
4. Spinach is a major component of many prepared food recopies in various human cultures and societies which make it an easy candidate for contaminations. Even though, this may lead spinach to harbor harmful bacteria, it has much to offer human beings in terms of nutrient bioavailability. Bioavailability is "the degree to which our bodies can absorb and utilize any given nutrient" (Thompson and Manore, 2009, p. 368). Conduct Internet research to find out:
   - The bioavailability of the various nutrients that spinach contains.
   - The factors that affect the degree of the bioavailability of a given nutrient such as calcium by the human body.

Independent Research (I): Common Sources of Foodborne Illness

According to the U.S. Centers for Disease Control and Prevention, on the average, every year "about 76 million people in the United States become ill from pathogens (bacteria, parasites, or viruses), or disease-causing substances (natural and/or synthetic chemicals), in contaminated food and drinking beverages. Of these people, about 5,000 die" (NIH Publication No. 07–4730 - May 2007). Harmful bacteria are a common cause of foodborne illnesses. Conduct Internet research to find out the common sources of foodborne illness and common symptoms. Use table 3 to record your findings.

Table 3: Sources & Symptoms of Foodborne Illness

Bacterium	Food Sources of Illness	Symptoms	Additional Observations
Escheria coli O157:H7			
Listeria monocytogenes			
Shigella			
Yersinia enterocolitica			
Clostridium botulinum			
Salmonella enteriditis			
Staphylococcus Aureus			
Campylobacter jejuni			

Independent Research (II): Professional Patient and Governmental Organizations and Agencies

Conduct Internet research to find out the main accountabilities and responsibilities of the professional, patient, and government organizations and agencies that are listed in Table 4. Also, look for the means by which each organization communicates information to its various constituencies. Finally, search for five more organizations with related responsibilities that you think are also important for every citizen to know about, and add them to Table 4.

Table 4: Organization's Accountabilities & Responsibilities

Organization	Accountability & Responsibilities	Headquarters	Communication Information
American Dietetic Association			
Center for Food Safety and Applied Nutrition			
Centers for Disease Control and Prevention			
Gateway to Government Food Safety Information			
Partnership for Food Safety Education			
U.S. Department of Agriculture			
U.S. Department of Health and Human Services			
U.S. Environmental Protection Agency			
U.S. Food and Drug Administration			
National Digestive Diseases Information Clearinghouse			

Conclusion:

The learning activities that are included in this article are designed to help students understand the importance of the critical role of hypotheses in the scientific method of inquiry, the orderly means of investigation to answer questions and solve problems in scientific situations. In addition, the activities are designed to link this concept to the bacterial contamination of food. Food contamination, foodborne illness, and health and safety have become an urgent matter in the United States. By engaging today's students in realistic learning activities, we create learning environments that promote active learning, critical thinking, collaborative learning, and knowledge creation--- habits that are urgently needed in the next generation of physicians, researchers, communicators and public policy makers. They will need this knowledge base, as they attempt to deal effectively with issues such as nutrition, health, safety, and wellness for their, and successive, generation(s).

Answers to Questions Raised in the Learning Activities:

A complete and detailed answers to all the questions raised in the learning activities in this paper are available electronically based on individual request by e-mail from the authors.

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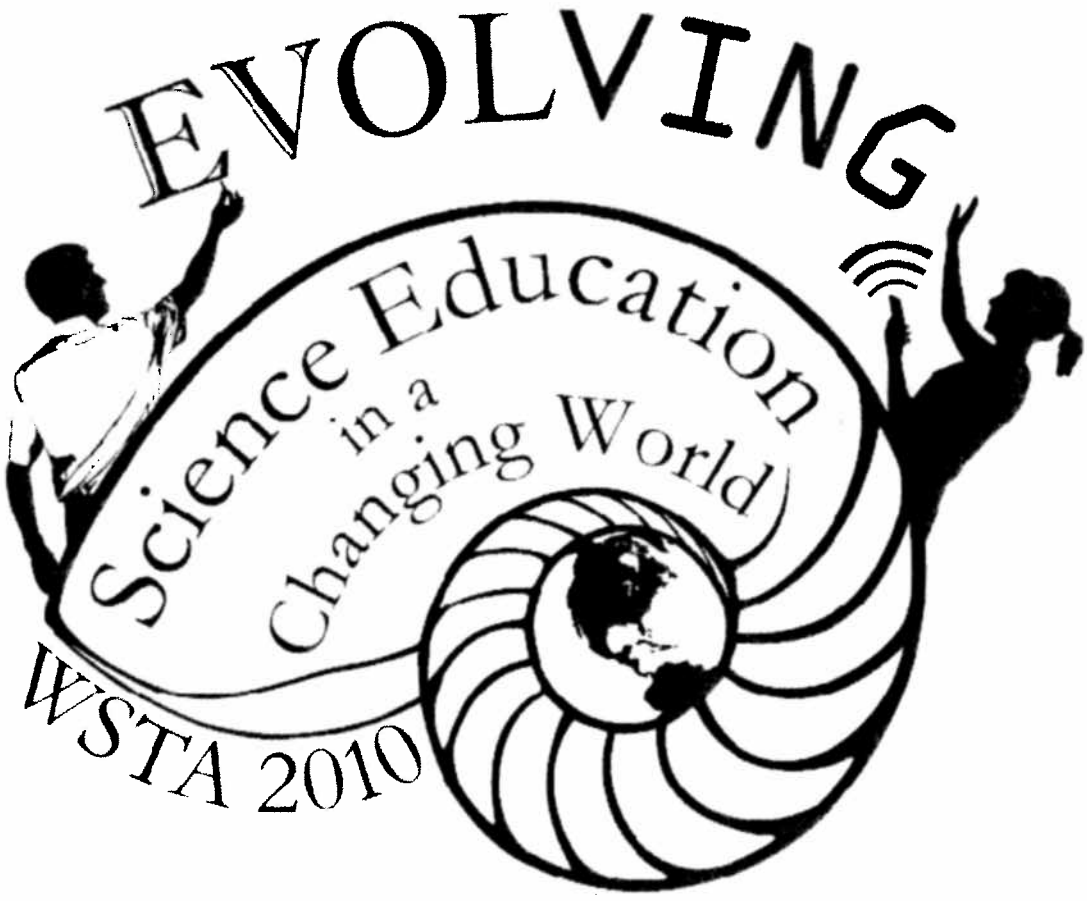

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