Effects of an Emerging Pathogen-Centered Lesson on Student Engagement and Perceived Lesson Effectiveness in the Advanced Placement Biology Classroom

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Abstract:

College Board (2015) redesigned the Advanced Placement (AP) Biology curriculum in Fall 2012 to emphasize critical-thinking skills and the interdisciplinary nature of science. With the redesign, a quarter of instruction is now dedicated to inquiry-based instruction, yet for many, lecturing and direct-instruction are still the preferred method of teaching. Lecturing is often known as an ineffective strategy for information retention, but direct-instruction has been shown to be effective at promoting student learning (Hattie, 2011; Peterson, 1979). A recent trend in education, like in the AP Biology curriculum redesign, is a shift towards student-centered learning as it has been found to be more engaging and impactful on learning than traditional teaching methods (Freeman et al., 2014; Smith, Sheppard, Johnson, & Johnson, 2005; Taraban, Box, Myers, Pollard, & Bowen, 2007); however, other studies contradict that conclusion (Mueller, Knobloch, & Orvis, 2015; Zahorik, 1996). This aim of this study is to determine the impact of an interdisciplinary, emerging pathogen-related lesson on student engagement and learning. The lesson incorporates information and research from the Center for Pre-Collegiate Education Training CATALySES program at the University of Florida. The program's theme of emerging pathogens and interdisciplinary research is reinforced in this AP Biology lesson. The lesson centers around student groups acting as molecular biologists, medical doctors, evolutionary biologists, and statisticians investigating plant and animal epidemics. Perceived lesson effectiveness and engagement will be measured with a 5-point scale Likert pre- and post-survey and will be coordinated with results from post-lesson interviews. The results will be used to determine which teaching strategy, direct-instruction or inquiry-based learning, garners the greatest student engagement and perceived student effectiveness.

Rationale:

The AP Biology redesigned curriculum was launched in Fall 2012 with an emphasis on overarching conceptual themes, known as "Big Ideas," and inquiry. College Board (2015), the non-profit organization responsible for developing AP curriculum, aimed to limit the breadth of the previous curriculum and instead focuses on deeper understanding and implementation of cross-discipline science practices. Reflected in the College Board's (2017) course overview, educators are now expected to spend a quarter of instructional time using laboratory and inquiry-based lessons.

Unfortunately, most educators continue to teach the way they have been previously taught. That previous method of instruction focuses primarily on lecturing or direct instruction. Lecturing is a method of presenting a significant amount of material in a limited amount of time and is often colloquially described as being a "sage on a stage." Lecturing, however, is often ineffective at maintaining attention and engagement over long periods of time. Direct instruction, another teacher-centered approach, is a preferred alternative to lecture as it elicits student feedback and engages students in a structured pattern of learning (Peterson, 1979). Per Hattie (2011), direct instruction rates in the top 16% of influences that impact student learning with an effect size of 0.60. While direct instruction still retains its value in the classroom, active-learning, especially through inquiry has been shown to be another effective tool in an educator's toolbox. Smith et al. (2005) showed that throughout all secondary and post-secondary levels of education, the greatest learning gains occurred when students were involved in student-centered lessons. Active-learning has been shown in undergraduate science courses, like those which AP Biology is based on, to be more positively impactful on examination scores than traditional lecturing (Freeman et al., 2014). Also, Taraban et al. (2007) have shown that active-learning is far more effective than traditional instruction, especially in factual recall and processes, both of which are often the focus of direct instruction. As with anything in education, data can often be conflicting, subject to several external factors, and prone to variability in student populations year to year. For example, research published by Mueller et al. (2015) found that active learning showed no significant difference between teacher-directed learning in contrast to research by Taraban et al. (2007). Zahorik (1996) also mentions a fear of hands-on activities being needless and wasteful of learning time when used inappropriately. Consequently, this proposal aims to determine the effectiveness of this lesson in relation to previous years of direct instruction to ensure it matches the potential that current literature and trends suggest.

In addition to lesson effectiveness, student engagement is also a key focus in this study. At Oviedo High School, AP Biology pass rates already far surpass the national and state averages at 88.1% (97/110) over a three-year period. Based on such success, the current pattern of direct instruction is effective, but engagement periodically lacks. Students in AP Biology have three primary motives for enrollment: 1) interest in science or biological-related fields, 2) attaining a higher grade-point-average, and 3) preparedness or earning credit for college. The effectiveness aspect of this proposal targets facets 2 and 3. The engagement aspect, however, can impact all three. Sutcliff (2011) revealed that content relevant to their lives inspires student motivation and learning. If students are interested and engaged in a topic, they will have a greater willingness to learn. The goal is to judge whether the predicted increase in engagement will also follow with effectiveness.

An indirect motive of this study is to provide exposure to potential biology-related careers, especially due to their growing interdisciplinary nature, and it targets those generally interested in science. A midway point in a student's high school career usually solidifies their interest in science (Aschbacher, Li, & Roth 2010). The redesign of the AP Biology curriculum perfectly matches the growing trend of interdisciplinary focuses in professional careers and academic research. Single-authored papers are becoming more of a rarity, and interdisciplinary team-based biological research often includes cooperation among statisticians, biologists, engineers, computer scientists, and even artists in order to convey their findings. In fact, during University of Central Florida's Summer Research Academy, it was predicted that the most coveted graduates in the future will be those who combine two degrees in order to act as a translator between the two disciplines (K. Teter, personal communication, June 2012). Because this lesson incorporates many roles, students will be exposed to several different sub-disciplines within and related to biological research, thereby increasing relevancy and concurrently engagement.

Measuring engagement and lesson effectiveness can be especially challenging. While used often at the collegiate level, teacher feedback surveys have rarely been solicited in the high school classroom. In regards to effectiveness, the prevailing fear is that high school students lack the maturity to accurately and appropriately assess the quality of their instruction, but Sutcliff (2011) showed that ratings by these students do show promise. This proposal uses a novel activity- and course-specific post-survey to assess effectiveness and engagement in response to the lesson.

Intervention:

The proposed intervention is to implement a collaborative, student-centered lesson utilizing interdisciplinary roles in response to an emerging pathogen outbreak. The scenarios will be as authentic as possible and will be based on real-life examples or possibilities wherever possible. There are two primary goal outcomes for this intervention: 1) Create an effective and 2) create an engaging lesson in contrast to lecture and direct-instruction.

Students will be divided into groups of either three or four and assigned one (or two in a group of three) of the following roles:

- **Molecular Biologist (Ph. D.):** Expert on macromolecules, cell signaling, and genetic aspects of the scenario.
- **Immunologist (M.D.):** Expert on anatomy, physiology, epidemiology, and immune response related to the outbreak.
- **Evolutionary Biologist (Ph. D.):** Expert on relatedness to other species, selective pressures, natural selection, and epidemiology of the disease.
- **Statistician (Ph. D.):** Expert on experimental design, explanation and presentation of data, and coordinating the research team.

Cases will be both animal- and plant-based in origin to emphasize the fact that pathogens aren't restricted to animals. This lesson also provides the opportunity to reinforce that plant pathogens can actually have a greater

impact on society, especially in terms of economics, than animal pathogens (J. G. Morris, personal communication, June 18, 2017). The following pathogens are topics for the research teams:

Pathogen	Scientific Name	Type	Disease	Location
Ebola virus	*Zaire ebolavirus*	Virus	Ebola	West Africa
Cholera	*Vibrio cholerae*	Bacteria	Cholera	Haiti
Late blight	*Phytophthora infestans*	Fungus	Late blight or potato blight	Ireland
Zika virus	*Zika virus*	Virus	Zika	Puerto Rico/Brazil
Salmonella	*Salmonella enterica*	Bacteria	Salmonellosis or Typhoid	Oregon
Huanlongbing	*Candidatus* Liberibacter *asiaticus*	Bacteria	Citrus greening	Florida

Each group will be given a pathogen, background information, and research findings to contextualize the outbreak. The provided resources will be keyed according to the role assumed by each member of the group. The information below provides a summary of an ideal case:

Zaire ebolavirus

Molecular Biologist (Ph. D.): The molecular biologist will be given a 3-5 page summary of research findings involving molecular mechanisms to the disease. Mechanisms can include cell-signaling pathways affected, virulence factors, genetic impact of the disease, lysogenic and lytic stages of the virus, and impacts on gene expression.

Immunologist (M.D.): The doctor will be given a 3-5 page summary of research findings showing signs and symptoms of the disease, quarantine measures, impact on the immune system, potentially susceptible hosts and vectors, vaccines, and treatment options.

Evolutionary Biologist (Ph. D.): The evolutionary biologist will be given a 3-5 page summary of research findings showing the phylogeny and genetic relatedness of the pathogen to previously characterized species, selective pressures potentially impacting its variability and mutation rate, tracing the course of the species throughout history. The evolutionary biologist will use that information to predict potential new infection locations and explore similar diseases to base potential treatment options from.

Statistician (Ph. D.): The statistician will be given a 3-5 page summary of research findings complementing the findings of the other three disciplines, such as summaries of disease spread, vaccination implementation procedures, or modeling and simulation tools. The statistician will aim to coordinate the understanding of the disease and will plan how to best implement treatment, vaccine, and public outreach initiatives.

Data Collection & Analysis:

Students will be given an informed consent to opt into participating in data collection. Students' perceived effectiveness of the lesson and engagement will be assessed with administration of a 30-question post-survey designed specifically for this activity and course. The survey will be administered at the conclusion of the unit in which the pathogen activity was implemented.

A post-lesson interview will also be held with five students preferably evenly divided between the two sections of the course and equal gender and grade level representation. The interview will be semi-structured and open-ended with the following questions (follow up questions are indented):

1. Why did you take AP Biology?
    a) What are your goal outcomes from the course?
    b) What impact does this have on your preparedness for the AP exam? Future aspirations?
2. How relevant did you find the lesson? Explain your thoughts.
3. How engaged were you in the lesson? What held or lost your attention?
4. Thinking back to why your took this course, would you like to see more lessons like this in the future, why or why not?
5. What recommendations or changes would you make for the future of this lesson and others?

Quantitatively, descriptive statistics will be carried out following the administration of the surveys. Qualitative data will be analyzed through in a deductive thematic fashion. For the engagement portion of this study, data will be coded using questions 2 and 3. For the effectiveness, data will be coded using questions 1, 4, and 5.

Connections to CATALySES:

Cases and roles were constructed based on real-life scenarios, roles, responsibilities, and resources provided by lectures and demonstrations in CATALySES program. The firsthand accounts of experimental design and data analysis by researchers on emerging pathogens were used as an inspiration for the roles the students will assume during this lesson. The action proposal intervention also emphasizes emerging pathogens as its primary theme. Each student group will be given a prior or current emerging pathogen, either animal or plant-based, that has caused significant impact to society, such as the Ebola epidemic in West Africa or citrus greening in Florida. The emerging pathogen topics the professors presented were greatly contextualized for their audience and they synthesized data much like in a literature review. Students will be doing the same in their presentations in this lesson. The professors did a great job weaving the scientific method, their thought processes and rationale, and their results in an easily understandable fashion. While this lesson doesn't focus on the implementation of the scientific method, especially since there will not be any experimentation, it does focus on critical thinking and effective presentation of findings.

Further trends evident during the CATALySES program were increased presence of interdisciplinary research, love of science, inquisitiveness, and willingness to disseminate their information to the public. Drs. Staras, Nelson, and Dean all had focuses on the interdisciplinary side of research through non-traditional roles in the research team (biostatistician in the case of Dr. Dean) or in translational research (getting the public on board in the case of Drs. Staras & Nelson). In nearly all the presenters, the love of science and curiosity were the forefront of their presentations. Especially captivating were Drs. Salemi, with his focus on evolutionary biology, Canella, with his focus on immunology, and Chen, with his work in proteomics. The previous three scientists, including many others, all were incredibly well-spoken and capable of reducing their complex research into a presentation tailored to their audience. And with their presentations, they all showed their commitment to informing the public, supporting education, and willingness to give back to those who supported them. This lesson hopes to embody the emotional connection of science the professors showed over the course of this program, hence the specific attention paid to student engagement.

Literature Cited:

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Permissions:

Consent to participate in the CPET CATALySES was already obtained by an administrator prior to acceptance into the program. Reaffirmation of that commitment from the principal and assistant principal is suggested to keep them informed of the action research and its intended outcomes.

Permission, in the form of informed consent, is required from parents and students. Parents and students will be informed of the purpose of the research, tasks and time required, benefits, and risks of the study. Parents and students will be provided with an informed consent form based on Society for Science and the Public's informed consent template.

Revision History:

June 29
Initial draft completed and submitted to the University of Florida's Center for Precollegiate Education & Training.

August 2
Revised Revised AP pass rate in Rationale to reflect to recent reporting of 2016-2017 results.
Revised specialists' roles and research excerpt volume.
Informed consent was created.
Activity- and course-specific survey was created.
Revised permissions.
Fixed typographical errors.

Informed Consent Form

Researcher: William J. Furiosi II
Title of Project: Effects of an Emerging Pathogen-Centered Lesson on Student Engagement and Perceived Lesson Effectiveness in the Advanced Placement (AP) Biology Classroom

I am asking for your voluntary participation in my action research project. Please read the following information about the project. If you would like to participate, please sign in the appropriate area below.

Purpose of the project: To determine the perceived effectiveness and engagement of a pathogen-centered lesson in AP Biology.

If you participate, you will be asked to: Complete a 30-question survey regarding the effectiveness of the lesson and your personal engagement. Four participants will also be asked to join in a post-lesson interview to gain further details about the lesson's utility.

Time required for participation: The 30-question survey should take no more than 10 minutes. The post-lesson interview will last no longer than 30-minutes.

Potential Risks of Study: Minimal risks are associated with this study. Students may feel uncomfortable assessing the quality of their instruction. Survey contributions will be anonymous, so the teacher will not be able to identify which student answered which question. The post-lesson interview will be moderated by someone other than Mr. Furiosi to minimize bias.

Benefits: The results of this survey can serve to improve future instruction in AP Biology and make students better prepared for success on their AP Biology exam, in college, and/or their future career. Student insights can help make future lessons more meaningful and more enjoyable if this lesson is effective and engaging. Likewise, if ineffective, this lesson will no longer be used.

How confidentiality will be maintained: Surveys will be conducted via eCampus and information will be collected anonymously. There is always the possibility of tampering from an outside source when using the internet for collecting information. While the confidentiality of your responses will be protected once the data is downloaded from the internet, there is always a possibility of hacking or other security breaches that could threaten the confidentiality of your responses. No personally identifiable information will be collected through the survey, so the only risk will be the acquisition of student responses.

If you have any questions about this study, feel free to contact:

Researcher: William J. Furiosi II **Email:** william_furiosi@scps.k12.fl.us

Voluntary Participation:
Participation in this study is completely voluntary. If you decide not to participate there will not be any negative consequences. Please be aware that if you decide to participate, you may stop participating at any time and you may decide not to answer any specific question.

By signing this form, I am attesting that I have read and understand the information above and I freely give my consent/assent to participate or permission for my child to participate.

Adult Informed Consent or Minor Assent Date Reviewed & Signed:_____
Participant Printed Name: _____ Signature: _____

Parental/Guardian Permission *(if applicable)* Date Reviewed & Signed:_____
Participant Printed Name: _____ Signature: _____

Lesson Engagement

No.	Question	Strongly disagree	Disagree	Neutral	Agree	Strongly agree
1	My attention was maintained for this activity more than a typical class.	1	2	3	4	5
2	Pathogens and disease are not relevant to me.	1	2	3	4	5
3	This activity exposes me to fields of science that I previously had not considered.	1	2	3	4	5
4	I researched at home or school beyond what was expected of me.	1	2	3	4	5
5	This activity showed me a genuine view of authentic science.	1	2	3	4	5
6	Pathogens and disease topics make learning about biology more interesting.	1	2	3	4	5
7	Scientific research is more difficult than I thought.	1	2	3	4	5
8	This activity makes me want to pursue a degree in the field of pathogens more than before.	1	2	3	4	5
9	Being able to discuss information with my classmates made learning more memorable.	1	2	3	4	5
10	I want to learn more about plant and animal pathogens.	1	2	3	4	5
11	I shared what I learned from this activity with friends and family.	1	2	3	4	5
12	I did not use my time wisely during this lesson.	1	2	3	4	5
13	I am more interested in biology after completing this activity.	1	2	3	4	5
14	It was difficult understand the goals of the activity.	1	2	3	4	5
15	I enjoyed this activity.	1	2	3	4	5

Lesson Effectiveness

No.	Question	Strongly disagree	Disagree	Neutral	Agree	Strongly agree
1	This activity improved my ability to analyze and evaluate scientific research.	1	2	3	4	5
2	This activity improved my collaboration and discussion skills.	1	2	3	4	5
3	I would like to see more activities like this implemented.	1	2	3	4	5
4	The activity was too difficult for my current academic abilities.	1	2	3	4	5
5	This activity helped to prepare me for the AP Biology exam.	1	2	3	4	5
6	I feel better prepared for college than I did before.	1	2	3	4	5
7	I feel better prepared for scientific research than I did before.	1	2	3	4	5
8	I cared more about the grade I would receive than the activity itself.	1	2	3	4	5
9	I found the articles too difficult to comprehend.	1	2	3	4	5
10	I have a better understanding of the interdisciplinary nature of scientific research.	1	2	3	4	5
11	Plant and animal pathogens are more of a concern than I previously thought.	1	2	3	4	5
12	This activity was more effective than lecturing.	1	2	3	4	5
13	I still have gaps in my knowledge after this activity.	1	2	3	4	5
14	The expectations of this activity were appropriate for the course.	1	2	3	4	5
15	This activity aligned well with the AP Biology curriculum.	1	2	3	4	5

LESSON PLAN

TITLE: Outbreak: Emerging Pathogens and the Quest to Overcome

AUTHOR: William J. Furiosi II, Oviedo High School, *Oviedo, FL*

KEY QUESTION(S):
What is the impact of plant and animal pathogens on society?
How do teams of researchers collaborate from various fields to solve a significant medical problem?
In what ways do emerging pathogens act as models for explaining molecular biology, epidemiology, statistics, and evolutionary biology?

SCIENCE SUBJECT: Biology

GRADE AND ABILITY LEVEL: Advanced Placement (AP) Biology for grades 10^{th} – 12^{th}.

SCIENCE CONCEPTS: Evolution, molecular biology, cell-signaling and interactions, epidemiology, immune system, natural selection, and data analysis.

OVERALL TIME ESTIMATE: Five 50-minute class periods.
Day 1: Introduction to the activity/research roles and review of historical context.
Days 2 & 3: Analysis and discussion of research article excerpts, and creation of presentations.
Days 4 & 5: Concluding remarks, reminders of overarching themes, and group presentations.

VOCABULARY:
Cholera

Gram-negative	Method of identifying bacteria based on a specific staining process. Gram-negative bacteria lack a thick peptidoglycan cell wall and have a lipopolysaccharide outer membrane.
Pandemic	Infectious disease outbreak that spans country boundaries and affects a large number of people.
Serogroup	Group microorganisms classified together based on similar cell-surface antigens.
Biotype	Group of individuals of a population with the same genotype.
Endemic	Native to a particular location or area.
Virulence	Level of organism's ability to cause disease.
Milieu	A person's social environment.
Antigen	Molecule or substance that causes an immune response within an organism, especially the production of antibodies.
Genomic island	Section of nucleotides within a bacterium's DNA that have been acquired by horizontal transmission and confers some benefit to the recipient.
Exotoxin	Secreted toxin produced by a microorganism; in contrast to an endotoxin, which is a toxin that is part of the organism's structure that is released when the cell is lysed.

Citrus Greening

Vector	An organism capable of carrying and disseminating a pathogen.
Psyllid	A small, plant-feeding insect; the Asian citrus psyllid is a vector for citrus greening disease.
Epidemic	Outbreak of disease in a localized area that affects a large number of people.
Hybridization	The process of combining DNA from two different sources.
Monoclonal antibodies	Antibodies that are uniform in composition and all target the exact same antigen.
Abate	To lessen or reduce the effects of.
Mortality	Death.
Instar	Incremental stage in the life cycle of insects as it reaches maturity.
Mottle	Splotches and patches of color.
Flush	First sprouting of new leaves.

Ebola

Hemorrhage	Uninterrupted, and often profuse bleeding.
Epidemic	Outbreak of disease in a localized area that affects a large number of people.
Recombinant	Novel, or new arrangement of DNA, especially in relation to the organism's parents.
Immunogenicity	Ability of a substance or organism to generate an immune response in a host.
Virion	Term describing a single virus particle outside of a host cell.
Interferon	Signaling protein, a cytokine, produced during an immune response, and is named for its ability to interfere with viral replication and reproduction.
Interleukin	Signaling protein, a cytokine, produced during an immune response, and is named for its ability to promote movement of leukocytes, or white-blood cells, to the site of an infection.
Reservoir	Host organism that maintains a pathogenic population without being harmed in the process, like bats or rodents for the bubonic plague.
Viral load	Number of viral particles per given volume of fluid inside an organism; a method of determining extent of infection or how infectious a virus is.
Endemic	Native to a particular location or area.

Potato Blight

Blight	Widespread browning, withering, and death of plant tissue in response to a pathogen.
Epidemic	Outbreak of disease in a localized area that affects a large number of people.
Fungus	A heterotrophic, eukaryotic organism, separate from plant and animal kingdoms, that cannot photosynthesize and must acquire their nutrients from the environment.
Oomycete	A fungus-like organism that was misidentified as a fungus, but is more closely related to diatoms and brown algae. The etymology of the name means, "egg fungus."
Gene silencing	Process of inhibiting expression of a gene either at the transcriptional or translational level. Examples include RNA interference, CRISPR, or DNA methylation.
Apoplastic	Related to the space outside the plasma membrane of a plant cell along the cell walls, that material can freely move.
Virulence	Level of organism's ability to cause disease.
Monoculture	When all members of a population are genetically identical; occurs often in agriculture in breeding the most effective organism, but also results in a greater susceptibility to disease.
Epiphytotic	The term for a plant-pathogen epidemic.
Disparate	Totally different and incomparable.

Salmonella

Virulence	Level of organism's ability to cause disease.
Polycistron	The organization of multiple genes in sequence that are all transcribed together in a single mRNA. Cistron is another term for "gene."
Microfold cell	Specialized cell type within the gastrointestinal tract responsible for initiating mucosal immunity.
Serovar	Variant of a microorganism of a particular species based on its cell-surface antigens.
Pathoadaptive	Adaptive ability of an microorganism to become more or less pathogenic.
Pathogenicity island	Section of nucleotides within a bacterium's DNA that have been acquired by horizontal transmission (genomic island) that confers pathogenicity to the organism.
Epidemic	Outbreak of disease in a localized area that affects a large number of people.
Node	Intersection or branch point that connects multiple locations.
Gram-negative	Method of identifying bacteria based on a specific staining process. Gram-negative bacteria lack a thick peptidoglycan cell wall and have a lipopolysaccharide outer membrane.
Epithelium	Lining of cells along an external surface of the body, like the skin or the intestines.

Zika

Epidemic	Outbreak of disease in a localized area that affects a large number of people.
Microcephaly	Congenital birth defect resulting in a smaller than normal head circumference. Micro- for "small" and -cephaly for "head."
Viremia	Presence of virus in the blood.
Dengue	Tropical disease transmitted by mosquitos, also known as breakbone fever.
Neural progenitor cells	Stem cells in the embryonic nervous system responsible for differentiating into structures like the brain, nerves, and spinal cord.
Elucidate	To make clear and apparent.
Guillain-Barre syndrome	A rare autoimmune disorder that results in partial or complete paralysis as the body attacks the myelination protecting nerve cells with no known cause or treatment.
Conjunctivitis	Inflammation of the conjunctiva, or the cellular lining of the eyelids and eyes.
Clade	A group of organisms sharing a common ancestor.
Papular rash	Skin inflammation that presents as small, red, raised bumps that often occurs as a symptom of an infectious disease.

LESSON SUMMARY: Students will engage in a role-play and discussion/analysis activity involving six major plant and animal pathogens. Each team will consist of one expert from the each of the following fields: evolutionary biology, molecular biology, immunology, and statistics. Students will first contextualize research excerpts and then synthesize their analysis into presentations.

STUDENT LEARNING OBJECTIVES WITH STANDARDS:
Due to the extensive interdisciplinary nature of this lesson, enduring understandings (EU) and science practices (SP) are utilized rather than essential knowledge statements. The enduring understandings are divided according to each expert's role in the activity. Note that many of these enduring understandings likely will overlap between disciplines, as well, depending on the article.

Learning Objectives:

1. Students will be able to evaluate scientific evidence in the fields of evolution, immunology, and molecular biology to address current trends in emerging pathogens and make predictions about the future of epidemics. **SP 5, SP 6**

    a. **Evolutionary Biologist:** EU 1.A, 1.B, 4.C
    b. **Molecular Biologist:** EU 2.B, 3.B, 3.D
    c. **Immunologist: EU** 2.C, 2.D, 2.E
    d. **Statistician:** EU 3.C., 3.E, 4.B

2. Students will be able to synthesize information across multiple biological disciplines and communicate findings to laypersons. **SP 1, SP 6, SP 7**

MATERIALS:

Essential

- 48 labeled manila envelopes; 8 for each group
  (Sample label shown to the right)
  - 4 for each team's roles per group
    - Molecular biologist
    - Evolutionary biologist
    - Statistician
    - Immunologist
  - 4 historical contexts per group

EPIDEMIC: Cholera
Molecular Biologist

- Laminated case studies separated by each research article excerpt
- Laminated vocabulary lists
- Worksheets (1 per student), Outline Drafts (1 per group) & rubrics (1 per group)

Supplemental

- 8 computers, laptops, or tablets; 2 for each group
- 24 vis-à-vis markers, 1 for each student

BACKGROUND INFORMATION:

Emerging pathogens are of great concern to researchers and health professionals as antibiotic effectiveness decreases and novel pathogens are discovered. Recent human infectious disease epidemics, like 2009 H1N1 influenza pandemic, the 2010 Cholera outbreak in Haiti, the 2014 Ebola outbreak in West Africa, and the 2016 Zika epidemic in Brazil and Puerto Rico, have shown how easily it is for disease to spread in the increasingly interconnected world. Furthermore, and less publicized, is the impact of plant pathogens on crop yield and the economies of the afflicted growing regions. Prime examples include the potato blight that led to the emigration of 1.5 million Irish in the middle 1800's and the citrus greening disease that has cost the state of Florida and citrus growers billions of dollars in failed crops and lost earnings.

While most of the impacts of emerging pathogens can be learned from the news, truly understanding this topic from a scientific point of view occurs through interdisciplinary research. Rather than solely focusing on the singular aspects of a pathogen, current trends in research now equally value a holistic approach. Researchers from a variety of disciplines, such as molecular biology, epidemiology, statistics, and evolutionary biology, all work to contextualize these new plant and animal scourges in order to more effectively combat them.

This lesson is designed to emphasize the impacts of emerging epidemics through an interdisciplinary lens. Students will engage in a team-based research analysis activity using excerpts from research journals that describe advancements against six plant and animal pathogens. The goal for this activity is to embody the inquiry-based instruction and reasoning encouraged by College Board and the AP Biology curriculum in the topics of evolution, molecular biology, immunology, and data analysis.

ADVANCE PREPARATION: Prior to implementation of the lesson, the teacher should have already completed extensive instruction in at least three of the following AP Biology topics: natural selection, evolution, cell-signaling, molecular biology, immunology, and data analysis. The teacher needn't cover all topics as this activity can serve as a good previewing opportunity for future lessons.

The teacher will want to have the following prepared before implementing the lesson:

1) **Prepare case information.** Print case information according to each expert's discipline. Each set of excerpts for the four roles should be printed separately and laminated if possible. All research excerpts should be placed into discipline-specific labeled manila envelopes to be handed out to each of the scientists. Four envelopes of historical context per group should also be made so all students can participate in the overview of the case.
2) **Arrange classroom for group activities.** Arrange the classroom so that students have the ability to discuss in groups, but also have the ability to write and use technology for preparing their presentations.
3) **Provide technology for presentation creation.** Have laptops, computers, or tablets available so students can work on their presentations in class and engage in additional research if desired.
4) **Make excerpts and primary sources available via the Internet.** Post each group's case information and primary sources to a learning-management system or class website for access at home.
5) **Personally review and annotate case information**. Annotate cases to identify key areas that the teacher would like students to focus on. For example, if a goal for the school year is to work on statistics, then emphasize *p*-values and standard error bars in many of the activity's graphs. Whereas if the focus is more on vocabulary, students should aim to establish meaning from context clues. Having an annotated key will help meet personal goals for instruction.

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

Day 1: **Introduction and Historical Context**

(5 minutes) **Introduce the background and disseminate cases**.

Set the stage for the activity by playing the following 2.5-minute video: Infectious Disease Virus Hunters. Inform students about the interdisciplinary role of research; rather than doing research solely in a laboratory setting, there is a collaborative effort between people working at the bench, in the field, and at the bedside. Announce that the students will be given one of six emerging pathogen cases where they will act as infectious disease hunters for both plants and animals. While there may be groans about plants, remind them that plants arguably have a much bigger impact on human health than animal pathogens. With the class already arranged in groups of four, hand out a case study to each group.

Special Consideration: Having more or less than 24 students.
If there are more than 24 students in the class, then a fifth student can be added to each group to serve as a principal investigator, or lead, for the team. If an additional group needs to be made,

duplicate a pathogen or devise a new case study. For less than 24 students, omit a pathogen or have students duplicate roles.

Possible Discussion Questions:

- What are some major infectious diseases that you are aware of?
- What kind of researchers are needed for understanding infectious disease?
- How does infectious disease threaten our existence?
- How are we making emerging pathogens more of an everyday reality?

(10 minutes) **Read historical context.**

Instruct students to read the historical context independently. As they read, encourage them to identify words they aren't familiar with (at least 3) and utilize their vocabulary chart included in their envelope. Highlight or underline important information for summarizing the history.

Term	**Original Sentence**	**Definition (Paraphrased/Context Clues)**

Annotations & Notes
▪ ▪ ▪ ▪ ▪

Possible Discussion Questions:

- What impacts did your disease have on society?
- Where did your pathogen originate and how is it transmitted?
- What type of pathogen are you studying, and how is it so effective?

(20 minutes) **Introduce research roles and read articles independently.**

Let students know that the content will be much more specific when they open their specific role case files, and in some circumstances, much more difficult to comprehend. Encourage students to reread, jot notes and questions off to the side with a vis-à-vis, and ask the teacher for help.

Students should open their specific roles envelopes and begin reading like they did previously with the historical context. As they read identify and define unknown words (at least three), annotate each research article excerpt. **The goal of their read should be extracting information for layman's understanding** and **understanding key claims made by figures**.

Special Considerations: Complexity of roles.
The roles have varying degrees of complexity based on the content area and the research article excerpts. The general difficulty is as follows (most to least challenging):

1. Molecular Biologist
2. Immunologist
3. Statistician
4. Evolutionary Biologist

It may be beneficial to assign certain students specific roles, or allow them to decide amongst themselves.

Term	**Original Sentence**	**Definition (Paraphrased/Context Clues)**

Annotations & Notes
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Possible Discussion Questions:

- Could you see yourself doing this kind of research? Why or why not?
- How well do you feel you understand the research you're reading? What could help you better understand it?

(12 minutes) **Discuss research with the team.**

At this point, students should discuss their notes with other members of their team. Some research roles have complementary information to others within the group. For example, one person may have the general summarized section of a paper, while another may have specific

results from the same paper. Some may simply have more generalized information to help others understand their more complex findings. Teams identify common trends and meanings from their excerpts to present to their peers. **The goal is to track the disease from inception, how it spread, evolved, and impacted society, and what current actions are being taken to understand or combat it.** They are also trying to **summarize their case into a two-sentence cause and effect statement**.

Possible Discussion Questions:

- How does your role's research contribute to the greater understanding of the program?
- What about other researcher's resources helps you understand your own?

(3 minutes) **Conclude and state homework.**

Let students know that they will have more time to process and discuss their research at home and tomorrow during class. Remind them that their goal is to trace students across the course of the epidemic by contributing information from their role's discipline. Have students take pictures of any vis-à-vis annotations made to excerpts and clear them for the next class. Students should continue to annotate and summarize articles at home if they need more processing time. Tomorrow, students should come to class with important and summarized information to contribute. Students should prepare clarifying questions for their group or their teacher as well.

Day 2: **Analysis and Discussion of Research; Presentation Creation**

(7 minutes) **Review, disseminate the six cases, and answer questions.**

Reemphasize that **the goal is to track the disease from inception, how it spread, evolved, and impacted society, and what current actions are being taken to understand or combat it.** They are also trying to **summarize their case into a two-sentence cause and effect statement**. Pass out the cases back to each group. At this point, students should have gone home and become more familiar with their own roles and come up with questions about their research. Answer any relevant questions in the whole group setting, and tell students more specific questions will be addressed personally when they begin their group discussions. **At the end of class, tell students they will need to submit an outline of their presentation at the end of class using the template provided**.

Remind students that they will need to cite ALL of their resources in APA format. Ideally, aim to cover APA style in a previous activity; if not, aim to allot at least 25 minutes to instructing proper APA style. Direct them to Purdue's Online Writing Lab (OWL) for citation templates. Students are expected to use parenthetical in-text citations in their presentations and have a references slide at the end of their slideshow.

(13 minutes) **Share summaries and annotations with group members.**

Students should continue collaborating about the key points from their research. They should use the notes they took from the day before and at home in order to contribute to the discussion.
Possible Discussion Questions:

- What are the key takeaways from your research excerpts?
- What shocking or interesting information did you learn from your research?
- Did you do any further research at home? If so, what did you find?

(10 minutes) **Establish cause and effect sentence.**

Revisit the cause and effect sentence they will use to conclude their PowerPoint presentation. The two sentences can be as long as necessary only if they do not become run-on sentences.

Below is a sample sentence for one of the six cases. It is not recommended to share this with the students since it's an example of a case used in this activity.

Example: Zika virus was discovered as a relatively harmless pathogen in Uganda beginning in 1947, but only recently did it evolve to become more virulent as it burgeoned into epidemic proportions in Brazil and Puerto Rico. Substantially more research is needed to understand and combat Zika, especially due to its ability to hide asymptomatically in large proportions of the population and its potential for significant birth defects like microcephaly.

Possible Discussion Questions:

- Where and how did your disease start?
- Where is the disease along its epidemic progression?
- How would you prioritize your key takeaways? Why is one takeaway more important than another?
- How can you take your key takeaways and turn them into a cause-and-effect statement?

(15 minutes) **Prepare outline for presentation.**

Inform students that they should be in the habit of outlining presentations similar to storyboards for movies. The goal is to make sure the presentation adheres to slideshow etiquette and progresses in a logical manner. Below is a template for the students to follow. **Remind them they do not need to have the labels on their final presentations.** Utilize the full worksheet at the end of this lesson for the presentation outline. At the end of their outline, they should include their draft cause and effect statement.

Slide Title:	Slide No.:
Slide Topic:	
Key Facts:	Figures:

(5 minutes) **Recap and collect presentation drafts.**

Have students take pictures of their presentation drafts so they know what to accomplish at home. For homework, students should work on their slides and come in tomorrow with their slides ready to input into their final presentation.

Day 3: **Analysis and Discussion of Research; Presentation Creation**

(10 minutes) **Reiterate expectations for presentations.**

Again, reemphasize that **the goal is to track the disease from inception, how it spread, evolved, and impacted society, and what current actions are being taken to understand or combat it.** They are also trying to **summarize their case into a two-sentence cause and effect statement**. Students will have 7 to 10 minutes for their presentations and time will be strictly kept. Also, all disciplines must be represented in the presentation. Citations must be included in the slides and references must be listed according to APA style at the end of the PowerPoint.

Take any clarification questions before allowing them to work on their presentations in class.

(35 minutes) **Assemble presentations.**

Allow students the chance to collaborate and assemble their presentation slides into a cohesive show. Students should be provided with computers or laptops (or advise to bring them from home if these resources are unavailable) in order to complete this activity. Encourage students to practice their presentations since pacing is often a challenge.

<u>Possible Discussion Questions:</u>

- How do you plan on organizing your presentation?
- What figures did you find most effective at conveying your message?
- What are the three most meaningful pieces of information you want to class to come away with?

(5 minutes) **Clean up and put away electronic devices.**

Remind students that tomorrow they will not have any time to assemble presentations, and they must be submitted to the teacher prior to the start of class via email or learning management system. Allow any last minute questions before the end of class as presentations will start promptly when class begins the next day.

Day 4: **Presentations & Recap**

(50 minutes) **Present student slideshows.**

Have group rubrics ready for assessing presentations. Pass out *Student Presentation Review & Takeaways* sheets so students can actively participate in the presentation and assess its quality. Presenters should remind the teacher when they approach their cause-and-effect statement so students have the chance to craft their own or the audience can take it home to complete.

Day 5: **Carry-over Presentations**

(20 minutes) **Present remaining student slideshows.**

Have group rubrics ready for assessing presentations. Pass out *Student Presentation Review & Takeaways* sheets so students can actively participate in the presentation and assess its quality. Presenters should remind the teacher when they approach their cause-and-effect statement so students have the chance to craft their own or the audience can take it home to complete.

(15 minutes) **Recap.**

Discuss overarching themes in evolution, molecular biology, statistics, and immunology. Review students cause-effect statements.

Possible Discussion Questions:

- What did you think of the activity? Are you interested in pursuing any of these fields of research?
- What concepts did you see in this activity that we have previously covered? What topics do you think we'll touch on in the future based on this lesson?
- How did evolution play a role in the emergence of each of these pathogens?
- In terms of molecular biology, how were these pathogens so effective at causing disease?
- Describe the role of a statistician in these research studies.
- What is common among most of these pathogens in regards to the immune system? What makes them so difficult to treat?
- Based on the approaches of each research time, what is the most effective method of combating these pathogens?
- How has your perspective of plant pathogens changed?

ASSESSMENT SUGGESTIONS:
Objective 1: Evaluate Evidence & Make Predictions
Excerpt Analysis: Student vocabulary, annotations, and notes can be checked for completion and via probing questions using the worksheet provided at the end of this lesson.
Student Presentation Review & Takeaways: Student annotations, notes, cause-and-effect statements, and evaluations of each slideshow via a worksheet maintains student accountability.

Objective 2: Synthesize Information & Communicate Findings

Pathogen Presentation: Assessed with an outline sheet and activity-specific presentation rubric.
Cause-and-Effect Statement: Assessed as a specific item in the Pathogen Presentation rubric.
EXTENSIONS:

Activities

Bacusmo, J. M., & Savage, K. (2012). *Bacteria Survivor Activity.* University of Florida. Retrieved from https://www.cpet.ufl.edu/wp-content/uploads/2016/07/Identification-of-Pathogenic-Islands-Using-Comparative-Genomics.pdf

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ADDITIONAL RESOURCES:

The following supplemental documents are used at varying stages in the activity:

Day 1 & 2: *Excerpt Analysis* (double-sided handout)
Day 3: *PowerPoint Presentation Outline*
Day 4 & 5: *Pathogen Presentation Rubric*
Day 4 & 5: *Student Presentation Review & Takeaways*

Excerpt Analysis

Historical Context

Term	Original Sentence	Definition (Paraphrased/Context Clues)

Annotations & Notes
Cause & Effect Statement:

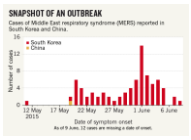
Case-Specific Research

Term	Original Sentence	Definition (Paraphrased/Context Clues)

Annotations & Notes

PowerPoint Presentation Outline

Directions: Each of these boxes act as a slide on your PowerPoint presentation. Briefly outline each slide included in your presentation. An example is provided. Be sure to represent each discipline and include the proper number of figures, in-text citations, and references.

Slide Title: Spread of the Disease **Slide No.:** 4 **Slide Topic:** Statistics **Key Facts:** **Figures:** ▪ Camels and bats believed to be the primary vectors (Zumla et al., 2015). ▪ Spread is confined to hospitals & extremely close contact is required (Zumla et al., 2015). ▪ No treatment for the disease ▪ Patients are often quarantined upon infection.	 (Butler, 2015)

Pathogen Presentation Rubric

Presentation

FOCUS	EXCEPTIONAL	PROFICIENT	UNACCEPTABLE
Information Accuracy	Information is represented accurately and fully describes: 1) disease origin, 2) spread, 3) evolution, 4) societal impact, and 5) current approaches. **10**	Information has minor errors of accuracy and is missing one of the five areas under **Exceptional**. **5**	Information has glaring errors of accuracy and/or is missing more than one of the five areas under **Exceptional**. **1**
Cause-and-Effect Statement	Cause-and-effect statement is two sentences and clearly describes the key cause and effect of the disease outbreak. **10**	Cause-and-effect statement is two sentences and somewhat describes the key cause and effect of the disease outbreak. **5**	Cause-and-effect statement is not two sentences and/or ineffectively or incorrectly describes the cause and/or effect of the disease. **1**
Effectiveness	Presentation is engaging, informative, relevant, and maintained the attention of the audience. **5**	Presentation lacked one of qualities under **Exceptional.** **3**	Presentation lacked more than one of the qualities under **Exceptional.** **1**
Balanced Representation	All four topics are discussed equally in time and content coverage. **5**	All four topics are covered in terms of content, but one is favored more than the others. **3**	One or more topics are missing. **1**
Figures Graphs, Data Tables, or Diagrams	Presentation includes and clearly describes **four** figures involving at least two of the four research disciplines. **5**	Presentation is missing one of the four required figures involving at least two of the four research disciplines. **3**	More than one of the required four figures are missing and/or figures are provided for only one discipline. **1**
Slide Etiquette	Appropriate text size (>20 pt. font), font color and background agreement, consistent style, and summarized bullet points are used. **5**	Presentation lacks one of the qualities under **Exceptional**. **3**	Presentation lacks more than one of the qualities under **Exceptional**. **1**
Time Limits	Presentation lasted 7 to 10 minutes. **10**	Presentation was over/under the 7 to 10 minute limit by less than a minute. **5**	Presentation was over/under the 7 to 10 minute limit by greater than one minute. **1**

APA Citations

FOCUS	EXCEPTIONAL	PROFICIENT	UNACCEPTABLE
Citation Requirement	Cites ALL research articles in the references section. **10**	One to two research article citations are missing in the reference section. **5**	Three or more research article citations are missing in the reference section. **1**

APA In-Text Citations Make in-text citation 10 pt. to save space if needed.	Correctly references all resources used for information or figures in the body of the presentation. **5**	In-text citations contain one to three error. *Examples*: Incorrect format of authors' names or punctuating 'et al.' incorrectly. **3**	In-text citations contain one to three error. **1**
APA Reference Style	References contain less than three errors, including, but not limited to, punctuation, indentation, or alphabetical order. **10**	References contain three to seven errors. **5**	References contains more than seven errors. **1**

IMPORTANT: Any information or figure that is not your own must have an in-text citation and a reference. Failure to use in-text citations and/or failure to include a references section will result in a 0 for all group members and a referral to discipline for plagiarism.

Student Presentation Review & Takeaways

FOCUS	EXCEPTIONAL	PROFICIENT	UNACCEPTABLE
Effectiveness	3	2	1
Logical Pacing & Sequencing	3	2	1
Slide Etiquette	3	2	1

Label each note according to the discipline:

E: Evolution **S:** Statistics **I:** Immunologist **M:** Molecular Biology

Annotations & Notes

Cause & Effect Statement:

HISTORICAL CONTEXT

Citrus Greening Disease (Huanglongbing) in Florida: Economic Impact, Management and the Potential for Biological Control

Author: Alvarez et al. (2016)
DOI: 10.1007/s40003-016-0204-z

Citrus greening disease or Huanglongbing (HLB) is a destructive vector-borne disease that affects all varieties of citrus and is currently threatening the existence of Florida (FL)'s citrus industry. While HLB was not discovered in FL until August 2005 [3], its vector has been present in the state since at least 1998 and has widely spread throughout the citrus producing regions of the state by 2003 [10]. The disease has spread rapidly and is now present in every citrus producing county in FL. In addition, HLB has also been found recently in or near urban areas in Brownsville and Houston, Texas, as well as Los Angeles, California. Outside FL, HLB is presently contained and has not yet affected other major citrus producing states. Other geographical areas suffering from HLB include citrus producing regions of Africa, Brazil, the Middle East, the Indian subcontinent and Southeast Asia [3].

There is no known cure for HLB, and the three major components of traditional HLB management include control of the insect vector through chemical or other means, aggressive removal of infected trees to reduce sources of inoculum within groves, and planting of disease-free nursery stock [9]. However, replanting trees and getting them into production without contracting HLB is costly and difficult even under intensive insecticide programs, making producers reluctant to follow the traditional three-tier system and instead choosing not to remove diseased trees if they remain productive.

Asian Citrus Psyllids (Sternorrhyncha: Psyllidae) and Greening Disease of Citrus: A Literature Review and Assessment of Risk in Florida

Author: Halbert & Manjunath (2004)

Asian citrus psyllid (*Diaphorina citri* Kuwayama, Sternorrhyncha: Psyllidae) may be the most serious pest of citrus in the world if any of the pathogens that cause citrus greening also are present. If none of the pathogens are present, the psyllids usually are minor pests. Citrus greening was reported in Brazil in July 2004 by Fundecitrus. This is the first report of the disease in the Western Hemisphere (Anon. 2004).

The Asian citrus psyllid causes damage to the crop primarily by transmission of the pathogen that causes greening, or "huanglongbing", which means "yellow dragon disease" in Chinese. Huanglongbing has been translated loosely as yellow shoot disease in English language publications because of characteristic yellow shoots caused by the disease. In addition to yellow shoots, the disease also causes mottling, chlorosis resembling zinc deficiency, twig dieback and reduced fruit size and quality. Fruit do not color properly, leading to the name greening. Fruit from diseased trees have a bitter taste. Other names include citrus vein phloem degeneration, and likubin, which means immediate withering disease. Australian citrus dieback, a disease of unknown etiology, is suspected to be caused by a similar psyllid-transmitted pathogen

(Broadbent 2000). Although the official name of the disease is huanglongbing (anon. 1996), we use the name "citrus greening disease" throughout this review because it is the name commonly used in the United States, and by our audience for this paper.

Citrus greening probably is the worst disease of citrus caused by a vectored pathogen. The dynamics, epidemiology, and molecular characteristics of the complex are poorly understood.

Incidence of Huanglongbing-Associated '*Candidatus* Liberibacter Asiaticus' in *Diaphorina citri* (Hemiptera: Psyllidae) Collected from Plants for Sale in Florida
Author: Halbert et al. (2012)
DOI: 10.1653/024.095.0312

What is clear is that movement of citrus plants across international borders over a period of several decades led to the dissemination of the pathogen and its vector. In Brazil, *D. citri* was introduced without the pathogens that cause HLB. *Diaphorina citri* was reported in Brazil in 1942 (Costa Lima 1942) and persisted in the absence of HLB for several decades. HLB was reported in Brazil for the first time in 2004 (Teixeira, et al. 2005) and has caused considerable damage to citrus there since its discovery. The discovery of HLB in Brazil was the first report in the Western Hemisphere. In Florida, HLB was discovered 7 years after the discovery of the psyllid, and the disease had spread into several counties by the time it was found (Halbert et al. 2008a, 2008b). In a very short period of time, HLB spread throughout the citrus-growing regions of the state.

An evaluation of plant genotypes for rearing Asian citrus psyllid (Hemiptera: Liviidae)
Author: Hall & Hentz (2016)
DOI: 10.1653/024.099.0320

Asiatic huanglongbing is one of the most serious diseases of citrus worldwide (Bové 2006). Also known as citrus greening or yellow shoot disease, Asiatic huanglongbing is putatively caused by a bacterium '*Candidatus* Liberibacter *asiaticus*' transmitted by the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) (Gottwald 2010). Huanlongbing can be a devastating citrus disease especially in sweet oranges and grapefruit, rendering trees so unhealthy that they retain little or no economic value. There is no known cure for huanglongbing. Asian citrus psyllid has spread from Asia to many areas around the world and was first found in the United States (Florida) in 1998 (Halbert & Manjunath 2004). Huanglongbing was discovered in Florida in 2005, is now endemic across this state's citrus growing regions, and has put the Florida citrus industry in serious jeopardy (Hodges & Spreen 2012; Hall et al. 2013)

Breeding a solution to citrus greening
Author: Vogel (2015)

CITRUS GREENING

Today, Florida is in the advanced stage of the epidemic. Every commercial grove in the state is assumed to be infected. Losses have been in the hundreds of millions. Entire groves have been ripped out or abandoned. Grapefruit production is at its lowest level since the Great Depression, excluding hurricane seasons. Orange production hasn't been this low in 40 years. The state's total citrus crop is less than half what it was in 2003-04.

Growers, the state and the federal Agriculture Department have combined to spend $263 million on research and prevention. (Florida growers agreed to tax themselves to raise $75 million so far.) Hundreds of scientists are looking for answers to the disease. Indeed, one way or another, every scientist at the University of Florida's Citrus Research and Education Center in Lake Alfred is working on greening, says center director Michael Rogers.

MOLECULAR BIOLOGIST

Asian Citrus Psyllids (Sternorrhyncha: Psyllidae) and Greening Disease of Citrus: A Literature Review and Assessment of Risk in Florida

Author: Halbert & Manjunath (2004)

Molecular approaches such as PCR and strain-specific DNA probes now have been used successfully to detect and differentiate *Candidatus* Liberibacter spp. both in infected plants and in psyllid vectors (Bove et al. 1993; Jagoueix et al. 1996; Tian et al. 1996). Unfortunately, detection is not always reliable. Sometimes trees with classic greening symptoms test negative with PCR (Toorawa 1998).

Molecular detection methods have been difficult to develop since the greening organism has not yet been cultured. Villechanoux et al. (1992) isolated total DNA from periwinkle plants infected with Indian greening, and digested it with restriction enzyme, *Hin*dIll. The digested DNA was cloned and the clones were screened by differential hybridization with DNA from both healthy and infected tissues. They identified three clones with 2.6, 1.9, and 0.6 kb inserts to be specific to the greening bacterium. The two larger clones reacted with all the Asian forms, but not with the African isolates, while the 0.6 kb clone reacted only with the Indian greening. Villechanoux et al. (1993) sequenced and analyzed the three greening specific clones. The larger 2.6 kb clone contained the genes of the nusG-rplKAJL-rpoBC operon, confirming the eubacterial nature of the greening organism at the molecular level. The 1 kb insert contained sequences for a bacteriophage-type DNA polymerase. The sequences from the 0.6 kb insert did not match anything in the database of known sequences.

UF research shows promise in finding cure for citrus greening

Author: DeWitt (2016)

The test tube in a tiny lab at the Lake Alfred Citrus Research and Education Center held dissolved citrus tissue. The fluid dripping into it from a pipette carried an enzyme that can snip genes like scissors, along with strands of RNA to guide the enzyme to precise genetic targets.

They are part of a gene-editing tool called CRISPR, which is so efficient it has been loosely compared to the "find and replace" function of a word-processing program. Nian Wang, the University of Florida professor leading this CRISPR research team at Lake Alfred, said the tool could cut decades from reaching the holy grail of citrus research — producing varieties immune from the disease that has devastated Florida's most iconic industry. "Come back in two or three years, and I'll show you a tree," Wang said.

The benefits of CRISPR are not just that it can eliminate genes that make citrus vulnerable to greening. It can replace them with other genes from the same plant, rather than from jellyfish or spinach, as has been the case with some previous genetically modified organisms. Mostly because CRISPR products don't borrow genes from other plants or animals, the U.S. Department of Agriculture ruled last spring that they won't face the same regulation as earlier generations of GMOs.

The race to stop a citrus plague

Author: Burke (2014)

The long-term solution to the citrus greening crisis has to involve a combination of blocking transmission from insects and promoting disease resistance in the tree. These issues and solutions necessarily arise in dealing with almost any invasive disease--management hinges on restraining transmission and susceptibility. The citrus industry sees no other choice but to embrace genetic modification of citrus trees to make them less susceptible to Liberibacter. As detailed in a July 2013 New York Times article, growers feel stuck between a rock and a hard place: Consumers want economical, U.S.-sourced citrus fruit and juice, but they don't want foods labeled as genetically modified. Regardless, a genetically engineered tree takes 7 to 10 years to grow and mature - time the industry does not have.

PCR detection of the two '*Candidatus'* liberobacter species associated with greening disease of citrus

Author: Jagoueix et al. (1996)

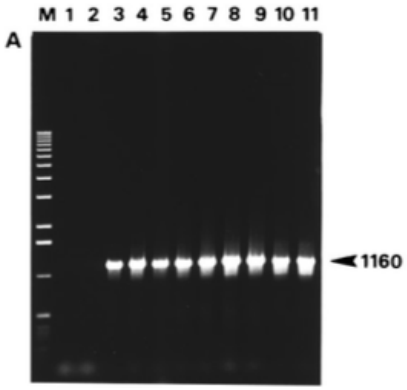


Figure 3. Electrophoresis on 0·7% agarose gel of DNA amplified with primers OI2c/OI1 from healthy sweet orange seedlings (lane 2) or sweet orange seedlings infected with *L. africanum* from South Africa (lane 3), Zimbabwe (lane 4), or *L. asiaticum* from China (lane 5), India (lane 6), Indonesia (lane 7), Nepal (lane 8), Philippines (lane 9), Ta¨iwan (lane 10) and Thailand (lane 11). M, 1 kb ladder (Gibco, BRL).

Characteristic 1160 bp bands were observed only with extracts from infected sweet orange leaves. … Using three different primer combinations, we have been able to specifically amplify 16S rDNA from the two '*Candidatus'* Liberobacter species known today: *L. asiaticum* and *L. africanum*. … Detection of plant pathogens by polymerase chain reaction in crude plant extracts is known to be difficult because of the presence of inhibitors of the PCR reaction. To avoid this, capture of pathogens by antibodies[14] prior to amplification has been largely used. However, in the case of the uncultured liberobacters of citrus greening disease, this method cannot be applied, as many serotypes of the bacterium have been shown to occur, and none of the monoclonal antibodies that have been produced so far, is able to react with all liberobacter serotypes.[15,16] This is why a procedure had to be developed to eliminate PCR inhibitors in the plant extracts.

EVOLUTIONARY BIOLOGIST

Asian Citrus Psyllids (Sternorrhyncha: Psyllidae) and Greening Disease of Citrus: A Literature Review and Assessment of Risk in Florida
Author: Halbert & Manjunath (2004)

The greening pathogens are thought to be highly fastidious phloem-inhabiting bacteria in the genus *Candidatus* Liberibacter. Although the bacteria have not been cultured for completion of Koch's postulates, circumstantial evidence points strongly to a bacterial disease agent because citrus greening symptoms abate temporarily when trees are injected with antibiotics (Buitendag & von Broembsen 1993; Lim et al. 1990; Su et al. 1986). The isolate from South Africa has been named *Candidatus* Liberibacter *africanus*, and the isolate from Asia has been named *Candidatus* Liberibacter *asiaticus* (Garnier et al. 2000). A subspecies of *Candidatus* L. *africanus*, *Candidatus* L. *africanus* subsp. capensis, has been de- scribed from the Western Cape Region of South Africa from *Calodendrum capensis* Thunb., a native South African plant. This subspecies also infects citrus (Garnier et al. 2000). Garnier et al. (2000) changed the generic name from Liberobacter to Liberibacter, following the International Code of Nomenclature of Bacteria, which states that since "bacter" is of masculine gender and "Liber" is of Latin origin, the connecting vowel should be an “i.”

Exclusion techniques reveal significant biotic mortality suffered by Asian citrus psyllid *Diaphorina citri* (Hemiptera: Psyllidae) populations in Florida citrus
Author: Qureshi & Stansly (2009)
DOI: 10.1016/j.biocontrol.2009.04.001

The objectives of our studies were to estimate the overall contribution of abiotic and biotic factors to natural mortality of *D. citri* cohorts or generations at different times and simultaneously identify the sources related to such mortality.
…
Spiders and insect predators in the families Blattellidae, Coccinellidae, Chrysopidae, Formicidae, Syrphidae, Anthocoridae and Miridae were observed on colonies or trapped in sticky barriers (Fig. 3). The Asian cockroach *Blattella asahinai* Mizukubo was the most often encountered species

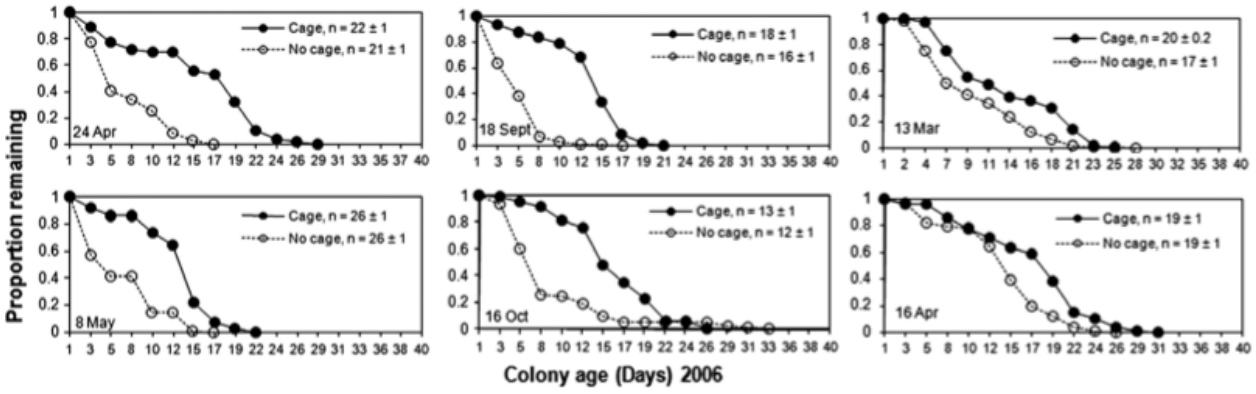


Fig. 2. Mean number of *Diaphorina citri* nymphs remaining through adult emergence in colonies established on shoots of *Citrus sinensis* that were fully caged or not caged (no exclusion barrier) to assess the impact of natural mortality factors on psyllid populations, 2006–2007. Dates in the figures indicate initiation of the experiment.

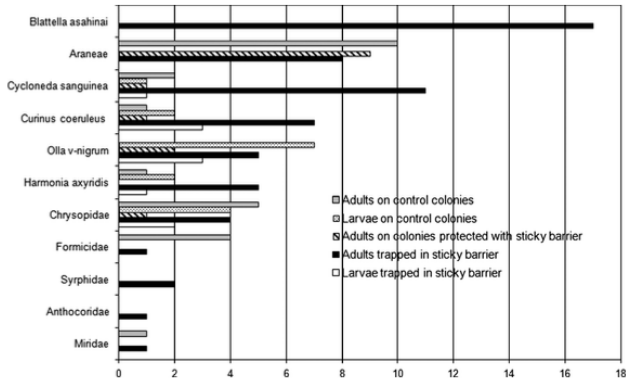


Fig. 3. Total number of predators observed on colonies of *Diaphorina citri* nymphs on *Citrus sinensi*s shoots or trapped in the sticky barriers used to protect colonies initiated on different dates, 2006–2007. Note: *Blattella asahinai* and *Araneae* data presented as adults include some juveniles not recorded as separate age class.

Impressive differences were observed in survival and consequently in estimated net reproductive rate between unprotected colonies compared to colonies protected with fine mesh cages. Sticky barriers and coarse mesh cages provided partial protection. These results suggested a leading role for predation among mortality factors impacting populations of *D. citri*.

Our results suggest that natural mortality factors, particularly predators and to lesser extent parasitoids, impose significant mortality on populations of *D. citri*. Surviving populations have proved sufficient to cause economically significant disease transmission. On the other hand, elimination of biotic mortality by intensive use of insecticides could require an impractical level of efficiency against increased incidence of pest and disease that might not be cost effective and would likely be inductive of secondary pest outbreaks. A preferable alternative is conservation and enhancement of biotic mortality through the use of selective insecticides, application methods and timings, coupled with inoculative and/or augmentative release of natural enemies.

Patterns of habitat use by the Asian citrus psyllid, *Diaphorina citri*, as influenced by abiotic and biotic growing conditions
Author: Pelz-Stelinksi et al. (2017)
DOI: 10.1111/afe.12197

During 2013, *D. citri* abundance was significantly greater in intermittently managed groves during winter compared with organic or conventionally managed groves. Furthermore, intermittently managed groves exhibited very little variability in psyllid abundance. Similarly, *D. citri* abundance was significantly greater during the winter of 2014 in organic and intermittently managed groves compared with conventionally managed groves

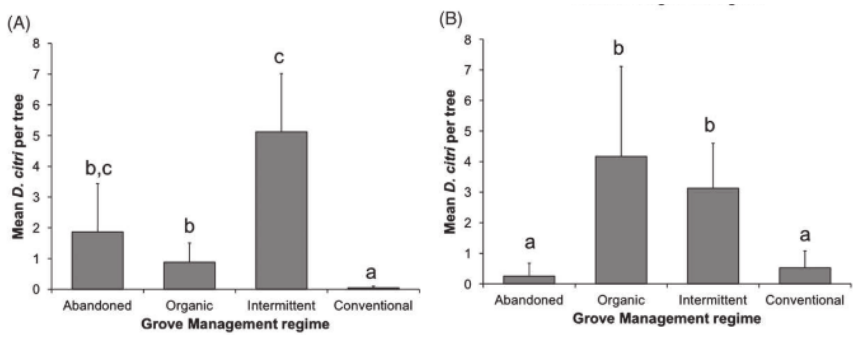


Figure 2. Distribution of *Diaphorina citri* during winter months (January to February) in groves sampled under different management regimes in (A) 2013 and (B) 2014

Groves with fewer than five insecticide applications per year (intermittent management) had significantly more *D. citri* during winter than groves under any other management regime. We would expect that, if insecticide applications alone were affecting *D. citri* abundance, unmanaged groves would harbor higher *D. citri* numbers, followed by intermittent and organic groves, whereas conventionally managed groves would have the lowest population abundance. A higher abundance in intermittently managed groves suggests that *D. citri* can overcome challenges of intermediate insecticide use within these groves. This is supported by the ability of *D. citri* to quickly develop resistance to various insecticides (Tiwari et al., 2011). Additionally, intermittent pesticide application likely reduced the density of natural enemies, which may explain why intermittently managed groves had greater psyllid densities than organic and abandonedgroves. The resurgence of pests after pesticide application is awell-known, unwanted side effect of pesticide application andhas been demonstrated in multiple systems

Diversity and Plasticity of the Intracellular Plant Pathogen and Insect Symbiont "*Candidatus* Liberibacter *asiaticus*" as Revealed by Hypervariable Prophage Genes with Intragenic Tandem Repeats

Author: Zhoi et al. (2011)

DOI: 10.1128/AEM.05111-11

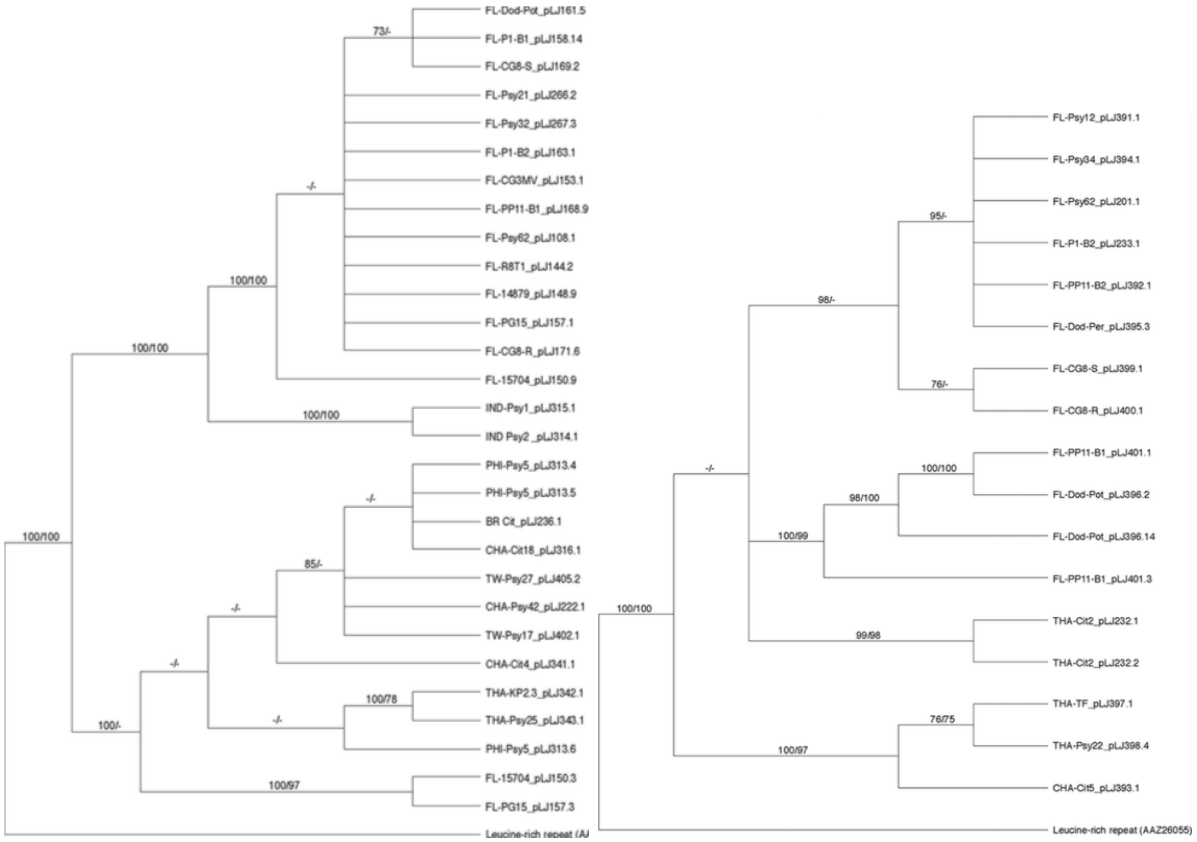


FIG. 3. (Left) Majority rule consensus tree based on maximum parsimony analyses of 29 amino acid sequences representing *hyv*I gene from global isolates of "*Candidatus* Liberibacter asiaticus." The leucine-rich repeat protein sequence from *Colwellia psychrerythraea* is used as the outgroup. **FIG. 4.** (Right) Majority rule consensus tree based on maximum parsimony analyses of 17-amino-acid sequences representing the *hyv*II gene from global isolates of "*Candidatus* Liberibacter *asiaticus*." The leucine-rich repeat protein sequence from *Colwellia psychrerythraea* is used as the outgroup.

Experiments to reveal the functions of the *hyv*I and *hyv*II genes are under way. … It is interesting to note that all the Florida isolates in this study contained both the *hyv*I and *hyv*II genes, while the isolates from other geographic origins only contained one or the other. The presence or absence of *hyv*I and/or *hyv*II genes varied with the origins of the "*Ca.* Liberibacter *asiaticus*"-infected samples. Nevertheless, at least one of them was always associated with the presence of a "*Ca.* Liberibacter *asiaticus*" bacterium. Based on the phylogenetic tree established on the basis of the *hyv*I gene variations, an early introduction of the disease agent (containing only the *hyv*I gene) may have occurred in India, and the introduction into the majority of the Florida "*Ca.* Liberibacter *asiaticus*" isolates occurred later. More recent introductions of the HLB disease into Florida may be evident, as two of the Florida "*Ca.* Liberibacter *asiaticus*" isolates were placed with good support in the *hyv*I clade with samples from Brazil, Philippines, Thailand, and China, including Taiwan (Fig. 3). In addition, the full *hyv*I genes from these international "*Ca.* Liberibacter *asiaticus*" isolates were identical, indicating a high possibility of the same source of "*Ca.* Liberibacter *asiaticus*" introduction into these four regions. The *hyv*II phylogenetic tree suggests that another major introduction of the HLB disease may have occurred from Thailand to Florida.

STATISTICIAN

Citrus Greening Disease (Huanglongbing) in Florida: Economic Impact, Management and the Potential for Biological Control
Author: Alvarez et al. (2016)
DOI: 10.1007/s40003-016-0204-z

While the true impact of HLB on citrus production in FL is difficult to determine, it is clear that disease has had a significant impact in the decline of citrus acreage in the state (Fig. 1). Total area planted in citrus peaked in 1995 at 330 thousand hectares (815 thousand acres) and declined slowly until 2001, when 290 thousand hectares (718 thousand acres) were planted throughout the state. There has been a marked acceleration in the decline of citrus acreage since that time, and by the 2012–2013 season, less than 198 thousand hectares (490 thousand acres) of citrus remained in production. Statewide citrus production has suffered a similar decline, going from 304 million boxes in the 1997–1998 season to 156 million boxes in the 2012–2013 season. All told, citrus acreage has decreased by 40 % and production by 49 % since their historical peaks, all of which occurred in the last 20 years.

There are a number of factors that have contributed to this observed decline in production area and productivity of citrus in FL. In addition to HLB, citrus canker, another adventive bacterial disease, has been affecting commercial citrus groves in FL since at least 1995 [4]. Recent changes in consumer preferences toward beverages with high sugar content may also be playing a part in the decline of FL's citrus production, of which a vast majority is destined for processed juice products.

Monetary estimates of the impact of HLB on FL citrus have begun to emerge in recent years. Hodges and Spreen [16] used a programming model of the global citrus market to estimate the price and quantity of orange juice that would have been observed in the absence of HLB and compare it with the actual price and quantity between the 2006–2007 and the 2010–2011 harvest seasons, and estimated a production loss of $1.7 billion over this 5-year period. Similarly, Moss et al. [24] developed estimates of lost producer and consumer surplus as a result of the current outbreak of HLB, and concluded that in the 2012–2013 season, consumers and producers lost an estimated $1 billion.

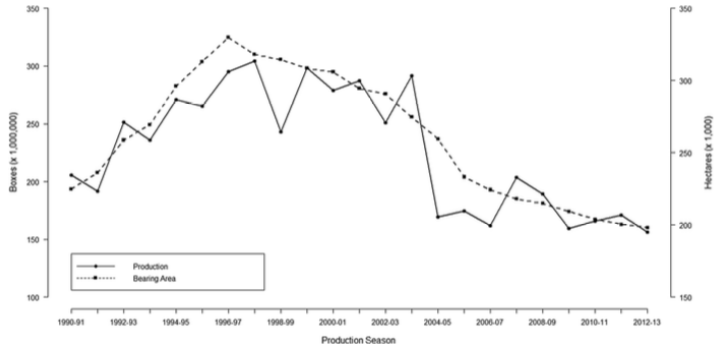


Fig. 1 Citrus production and bearing hectarage in Florida, 1990–1991—2012–2013.

USDA Invests $13.6 Million in Citrus Greening Research

Author: USDA (2017)

Huanglongbing (HLB) is currently the most devastating citrus disease worldwide. HLB was first detected in Florida in 2005 and has since affected all of Florida's citrus-producing areas leading to a 75 percent decline in Florida's $9 billion citrus industry. Fifteen U.S. States or territories are under full or partial quarantine due to the presence of the Asian citrus psyllid (ACP), a vector for HLB.

Since 2009, USDA has invested more than $400 million to address citrus greening, including more than $57 million through the Citrus Disease Research and Extension Program since 2014. Awards for grant applications submitted in FY 2016 include:

- Clemson University, Clemson, South Carolina, $4,274,523
- Regents of the University of California, Riverside, California, $5,112,000
- Iowa State University, Ames, Iowa, $2,476,099
- USDA Agricultural Research Service (ARS), Athens, Georgia, $1,821,197

Incidence of Huanglongbing-Associated '*Candidatus* Liberibacter *Asiaticus*' in *Diaphorina citri* (Hemiptera: Psyllidae) Collected from Plants for Sale in Florida

Author: Halbert et al. (2012)
DOI: 10.1653/024.095.0312

Between Aug 2005 and Aug 2009, we analyzed 1,186 psyllid samples. Forty-four of Florida's 67 counties were represented in the samples (Fig. 1). Although counties were not sampled evenly, there was a fair representation from many Florida counties.

Samples of *D. citri* were obtained from a variety of venues, including general retail nurseries, large discount garden centers, commercial citrus nurseries (nurseries that propagate citrus), and Foundation Budwood Blocks (Fig. 2). There was a marked decrease in numbers of psyllid samples collected from commercial citrus-propagating nurseries and Foundation Budwood Blocks in 2008, when the new Florida regulations became mandatory. These new regulations required citrus to be propagated in secure facilities, protected from psyllids. Frequency and intensity of inspections for commercial citrus nurseries also increased. *Murraya exotica* production was included in the regulations (FDACS/DPI 2008).

About 88% (1,031) of the *D. citri* samples were collected from citrus plants (Fig. 3). Another 11% (125) were from *M. exotica*, and 1.5% (18) were obtained from other citrus relatives. Twelve samples had no host data. Most of the *M. exotica* samples were collected in 2005-2006. Early in 2008, *M. exotica* became unavailable commercially in Florida. The new regulations made this plant prohibitively expensive to propagate.

Overall, about 9.7% of the 1,186 tested *D. citri* samples collected from plants for sale were positive for Liberibacter *asiaticus* (Las). Numbers of *D. citri* samples submitted and percentages of positive samples varied by year (Fig. 4).

Murraya exotica played an important role in the distribution of *D. citri* throughout Florida (Halbert et al. 2002). After the insects were distributed widely in Florida, little attention was paid to psyllid infestations on *M. exotica* plants for sale prior to the discovery of HLB in late summer, 2005. Over 15% (12 of 78) of *D. citri* samples from *M. exotica* plants for sale tested in 2005 and 2006 were positive for Las. An unknown number of pots of *M. exotica* that might have carried Las-positive psyllids left south Miami-Dade County prior to the implementation of new regulations in 2008, when the plants were not produced anymore. It is possible that some Las-positive psyllids on potted *M. exotica* were distributed widely through efficient networks maintained by large discount retailers.

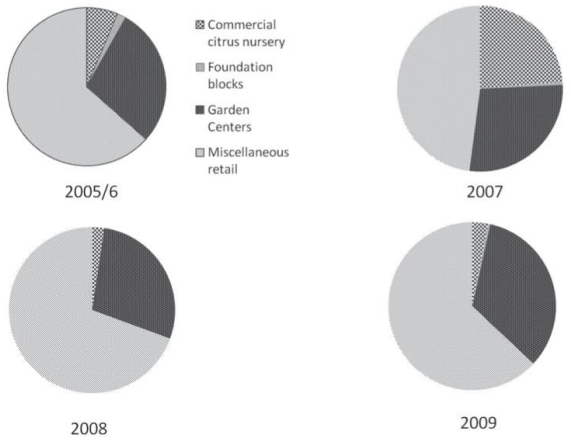


Fig. 2. Relative numbers of samples of *Diaphorina citri* Kuwayama collected on plants for sale at various types of nursery businesses in Florida (2005-2009). Legend begins at the 12:00 position and proceeds clockwise. Total numbers of samples by year: 2005/6 – 501, 2007 – 338, 2008 – 85, 2009 – 262.

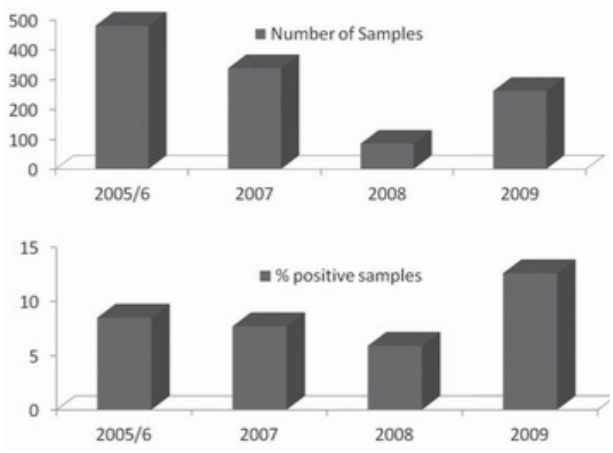


Fig. 4. Numbers of samples (top) and percent samples positive for '*Candidatus* Liberibacter asiaticus' (bottom) of *Diaphorina citri* Kuwayama collected from plants for sale in Florida (2005-2009).

Global climate suitability of citrus huanglongbing and its vector, the Asian citrus psyllid, using two correlative species distribution modeling approaches, with emphasis on the USA
Author: Narouei-Khandan et al. (2016)
DOI: 10.1007/s10658-015-0804-7

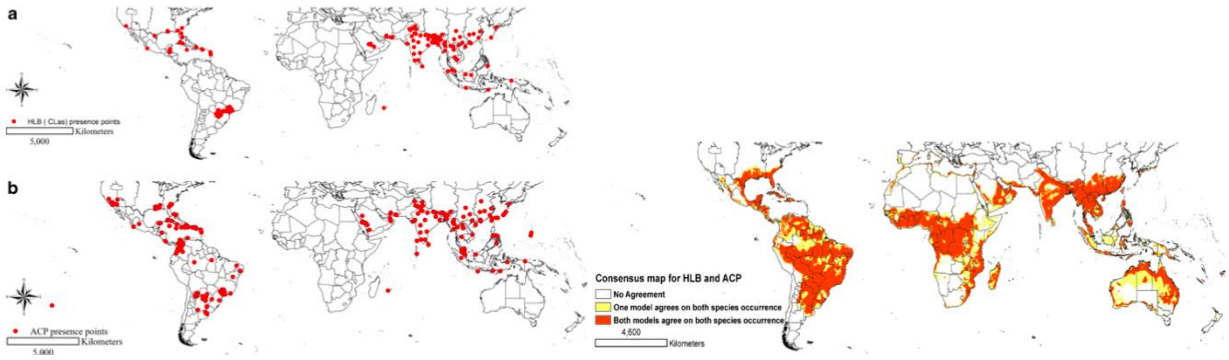


Fig.1 (Left) Current global distribution of citrus Huanglonbing, HLB, caused by *Candidatus* Liberibacter *asiaticus* (Las) (a) and Asian Citrus Psyllid, ACP (b). Occurrence of HLB and ACP in the USA were not included in the models. **Fig.6** (Right) Consensus model showing the hotspot areas where one or two models (MaxEnt and SVM) agree on the probability of both citrus HLB caused by Las and the ACP occurrence.

The consensus model showed that central and southeastern Africa, north and eastern Australia, southern China, India, most parts of South America, Florida, Georgia, North and South Carolina, Texas, Louisiana, and small coastal areas in California were potential hot spots of Las and ACP.

IMMUNOLOGIST

Citrus Greening Disease (Huanglongbing) in Florida: Economic Impact, Management and the Potential for Biological Control
Author: Alvarez et al. (2016)
DOI: 10.1007/s40003-016-0204-z

While the favored method for controlling the insect vector of HLB is through insecticide use, there are a number of organisms that are known to be effective predators or parasites of the vector. One of these organisms, the parasitoid wasp *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophiadae), was chosen by the FL Department of Agriculture and Consumer Services (FDACS) Division of Plant Industry (DPI) to be reared for mass release among citrus producers in the state [23, 33].

HLB is caused by phloem-restricted bacteria of the *Candidatus* Liberibacter group, of which at least three different species are known to occur: an Asian strain (*Candidatus* Liberibacter *asiaticus*), an African strain (*Candidatus* Liberibacter *africanus*) and a strain found only in Brazil (*Candidatus* Liberibacter *americanus*). These three species of bacteria can be transmitted by two species of citrus psyllids: Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) and African citrus psyllid, *Trioza erytrae* (Del Guercio) (Hemiptera: Triozidae), although the bacterial and vector species are geographically isolated and only overlap in a few locations such as the islands of Mauritius and Reunion in the Indian Ocean [3].

In FL's HLB outbreak, *D. citri* is the only vector present and *Ca.* L. *asiaticus* is the bacterial species identified as the causal agent [3, 15]. Trees infected with HLB first exhibit yellow shoots and blotchy mottle leaves reminiscent of nutrient deficiencies, and fruits on affected branches are small and lopsided, produce aborted seeds, do not change color properly and drop prematurely [3]. As the disease progresses, the tree loses productivity as entire branches, and then the whole tree, die. If the disease is widespread, citrus trees may live for only 5–8 years and never produce usable fruit [36].

The Asian citrus psyllid depends heavily on new flush (newly produced leaves) from citrus species for survival and reproduction. *D. citri* females deposit their eggs only on young tissue and can lay up to 800 eggs during their lifetime. Eggs hatch in 4 days or less, and the larval stage consists of five instars that are completed in 15 days or less, after which mature adults emerge. The length of the life cycle is dependent on temperature, but normally lasts between 15 and 47 days [21]. While *D. citri* is generally considered poor fliers and can only fly over distances of one mile in several days [2], their long range dispersal is aided by prevailing winds and unintended human transport of host plant material. For instance, it is believed that natural dispersal alone—aided by prevailing winds—is responsible for the spread of HLB-carrying psyllids from urban coastal southeast FL to commercial citrus groves in Hendry county, which is located more than 50 km away on the opposite end of the FL Everglades, an area with no known psyllid hosts [11, 13]. It is also believed that *D. citri* was able to establish widely in FL as a result of shipments of the ornamental bush orange jasmine (*Murraya paniculata*) infested with Asian citrus psyllid nymphs and adults that were grown in southern Miami-Dade County and were later sold in discount chain stores throughout the state [12].

Asian Citrus Psyllids (Sternorrhyncha: Psyllidae) and Greening Disease of Citrus: A Literature Review and Assessment of Risk in Florida
Author: Halbert & Manjunath (2004)

Citrus greening is a very destructive disease. A survey conducted over an 8-year period in Reunion Island, France indicated that 65% of the trees were badly damaged and rendered unproductive within 7 years after planting (Aubert et al. 1996). In Thailand, citrus trees generally decline within 5-8 years after planting due to citrus greening (Roistacher 1996). Roistacher (1996) showed that groves must live for a minimum of about 10 years in order to make a profit. Infected trees are stunted and sparsely foliated. The symptoms can resemble nutritional stress, especially zinc deficiency symptoms on recent growth; however, a more diagnostic mottle usually occurs on slightly older leaves that resembles symptoms of luteoviruses in dicots (e.g., Potato leafroll luteovirus). The mottle differs from nutrition-related mottling in that greening induced mottling usually crosses leaf veins, whereas nutrition-related mottling usually occurs between or along leaf veins. Off-season bloom, fruit drop, and twig dieback are other symptoms. Fruit are small, lopsided, hard, and have a bitter flavor. Seed abortion is common (Capoor et al. 1974). Citrus greening disease may predispose plants to other pest problems such as the citrus longhorned beetle, *Anoplophora chinensis* Forster (Aubert 1990b). A combination of citrus greening, citrus longhorned beetle, and as-sociated *Phytophthora* fungi are common in advanced citrus greening epidemics (Aubert 1990b).

Toorawa (1998) attempted to compile global infection statistics. He estimated 50 million trees infected in south and southeast Asia, three million trees infected in Indonesia, and ten million trees infected in Africa. In India and Saudi Arabia, there has been a marked decline in citrus industries as a result of citrus greening disease.

Asian Citrus Psyllid RNAi Pathway – RNAi evidence
Author: Taning et al. (2016)
DOI: 10.1038/srep38082

In this study, genome and functional analysis were performed to verify whether the RNAi core genes are present in the Asian psyllid genome and if the RNAi machinery could be exploited to develop a management strategy for this pest. Analyses of RNAi-related genes in the Asian citrus psyllid genome showed an absence of sequences encoding R2D2, a dsRNA-binding protein that functions as a cofactor of Dicer-2 in *Drosophila*. Nevertheless, bioassays using an *in Planta* System showed that the Asian citrus psyllid was very sensitive to ingested dsRNA, demonstrating a strong RNAi response. A small dose of dsRNA administered through a citrus flush was enough to trigger the RNAi mechanism, causing significant suppression of the targeted transcript, and increased psyllid mortality.

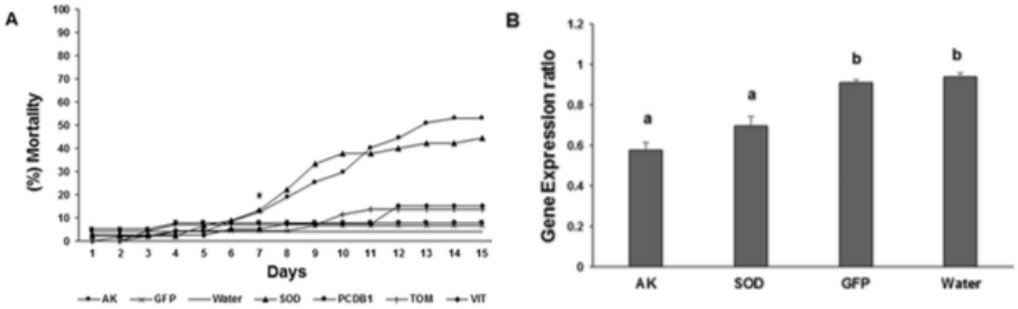


Figure 4. Ingestion of ACP-speci c dsRNAs induced insect mortality and gene suppression. (**A**) Adult ACP were fed on citrus ushes treated with dsRNAs targeting five ACP genes and controls treatments (dsGFP and water). ACP mortality was monitored over a 15 days period. e asterisk indicates the first day where cumulative mortality was observed on flushes treated with dsAK or dsSOD, which showed statistical differences ($P < 0.05$) compared to the controls (dsGFP and water). (**B**) Adult ACP showed reduced levels of AK (Arginine kinase) and SOD (Superoxide Dismutase) mRNAs 5 days post feeding (dpf) on a ush treated with dsAK or dsSOD. Feeding on flushes treated with dsGFP or water did not alter target gene expression in psyllids. Bars represent the standard deviation. Different letters indicate statistically significant differences ($P < 0.05$).

Asymptomatic spread of huanglongbing and implications for disease control
Author: Lee et al. (2015)
DOI: 10.1073/pnas.1508253112

We have shown experimentally that the latency period from new infection by infected adult psyllids to infectiousness in young flush is less than 15 days. In subsequent experiments, most of the plants that were colonized by psyllids developed HLB symptoms, but those plants that did not have nymphs usually failed to develop disease. These intriguing observations have not been quantified and deserve further study. Using the latency period information in a model where feeding by infected adults on young flush subsequently infects nymphs already on the flush, we showed that entire groves can become infected in a few months. The resulting infected trees can all be asymptomatic, and can become home to on the order of 12,000 psyllids, a large fraction of which are infected, during a single flush period. Because trees do not tend to show symptoms for anywhere from 1–2.5 y, and possibly longer, after initially becoming infected, emphasis must be placed on ongoing surveillance and control of psyllids

Our simulations indicate that 75% of reductions in the psyllid population as a result of control strategies carried out during all flush periods in a year can delay the appearance of symptomatic trees by at least 240 d, and by 1 y or more in many instances, beyond what would ensue without such control. If it is feasible to attain a 90% psyllid population reduction compared with the population without psyllid control, groves could be producin ruit free of HLB for 2+ y beyond the time of symptom onset in uncontrolled groves. Such reductions from spraying adult psyllids have been demonstrated in Brazil, for example (28).

Insecticidal Suppression of Asian Citrus Psyllid Diaphorina citri (Hemiptera: Liviidae) Vector of Huanglongbing Pathogens

Author: Qureshi et al. (2014)
DOI: 10.1371/journal.pone.0112331

A combined effect of nymphal and adult suppression in response to sprays of 23 insecticides representing 9 modes of action (MoA) groups and 3 unknown MoA provided more than 90% reduction of adult D. citri over 24–68 days.

Diaphorina citri populations respond rapidly to selection pressures due to high fecundity and short generation times, so any insecticide application selects for resistance. Some degree of resistance to key insecticides has already been documented in ACP populations in Florida [76]. Therefore, it is prudent to use a particular MoA only once a year.

Research has shown that 4–7 sprays targeting ACP in citrus orchards with close to 100% HLB incidence significantly increased yields, but were not always cost effective when combined with foliar nutrient sprays to mitigate effects of HLB [80]. Slowing spread of HLB under low incidence conditions would have more far reaching implications but has not be quantitatively evaluated in the US, although aggressive vector control coupled with rogueing of symptomatic trees is purported to be successful on large citrus plantations in Brazil [81]. A further consideration is the effect of resident ACP populations on the sustainability of new citrus plantings in the face of HLB for which economic analysis is not yet forthcoming.

At least one and preferably two aerial or ground applications of broad- spectrum insecticides during the “dormant” (winter) season when most mature trees are not flushing has been shown to provide significant reduction in ACP and need for insecticides into growing season as well as conserving biological control.

HISTORICAL CONTEXT

Emergence of Zaire Ebola Virus Disease in Guinea

Author: Baize et al. (2014)
DOI: 10.1056/NEJMoa1404505

Outbreaks caused by viruses of the genera ebolavirus and marburgvirus represent a major public health issue in sub-Saharan Africa. Ebola virus disease is associated with a case fatality rate of 30 to 90%, depending on the virus species. Specific conditions in hospitals and communities in Africa facilitate the spread of the disease from human to human. Three ebolavirus species have caused large outbreaks in sub-Saharan Africa: EBOV, *Sudan ebolavirus,* and the recently described *Bundibugyo ebolavirus*.[1,2] Epidemics have occurred in the Democratic Republic of Congo, Sudan, Gabon, Republic of Congo, and Uganda.

On March 10, 2014, hospitals and public health services in Guéckédou and Macenta alerted the Ministry of Health of Guinea and - 2 days later - Médecins sans Frontières in Guinea about clusters of a mysterious disease characterized by fever, severe diarrhea, vomiting, and an apparent high fatality rate. (Médecins sans Frontières had been working on a malaria project in Guéckédou since 2010.) In Guéckédou, eight patients were hospitalized; three of them died, and additional deaths were reported among the families of the patients. Several deaths were reported in Macenta, including deaths among hospital staff members.

Ebola Virus Disease

Author: Jin (2014)
DOI: 10.1001/jama.2014.13759

The current 2014 outbreak of Ebola virus disease in West Africa is the largest outbreak of the disease in history. As of October 2, 2014, there have been more than 3000 deaths from the disease. Currently, the mortality rate of Ebola virus disease is estimated to be about 50%, meaning about half of infected patients recover and the other half die of the disease. Aside from supportive care in the hospital (such as giving fluids and nutrition to patients when needed), there is no specific treatment for Ebola virus disease. No vaccine is currently available.

The countries most affected by the current Ebola virus disease outbreak are Sierra Leone, Guinea, Liberia, and Nigeria, all in West Africa. A major reason why the outbreak is worse than previous ones in Central Africa is because this outbreak has involved more urban areas. Also, many infected patients are cared for in hospitals or at home by health care workers who do not have access to proper protective equipment such as gloves, gowns, and eye goggles. In addition, the African ritual of washing deceased bodies at funerals in preparation for burial increases the amount of direct contact with Ebola victims, which increases the chance of family members becoming infected.

EBOLA

Adapted from a Centers for Disease Control and Prevention infographic.

Ebola Virus Disease in West Africa — The First 9 Months of the Epidemic and Forward Projections
Author: WHO Ebola Response Team (2014)
DOI: 10.1056/NEJMoa1411100

The epidemic began in Guinea during December 2013,[2] and the World Health Organization (WHO) was officially notified of the rapidly evolving Ebola virus disease (EVD) outbreak on March 23, 2014. On August 8, the WHO declared the epidemic to be a "public health emergency of international concern."[3] By mid-September, 9 months after the first case occurred, the numbers of reported cases and deaths were still growing from week to week despite multinational and multisectoral efforts to control the spread of infection.[1] The epidemic has now become so large that the three most-affected countries — Guinea, Liberia, and Sierra Leone — face enormous challenges in implementing control measures at the scale required to stop transmission and to provide clinical care for all persons with EVD.

Because Ebola virus is spread mainly through contact with the body fluids of symptomatic patients, transmission can be stopped by a combination of early diagnosis, contact tracing, patient isolation and care, infection control, and safe burial.[1] Before the current epidemic in West Africa, outbreaks of EVD in central Africa had been limited in size and geographic spread, typically affecting one to a few hundred persons, mostly in remote forested areas.[4] The largest previous outbreak occurred in the districts of Gulu, Masindi, and Mbarara in Uganda.5 This outbreak, which generated 425 cases over the course of 3 months from October 2000 to January

2001,[6] was controlled by rigorous application of interventions to minimize further transmission - delivered through the local health care system, with support from international part- ners.[5,7,8]

Ebola virus vaccines: Where do we stand?
Author: Pavot (2016)
DOI: 10.1016/j.clim.2016.10.016

ZEBOV is responsible for the recent outbreak in West Africa (2013–2016), the largest outbreak ever recorded since the virus was discovered in 1976, with a total number of 28,616 confirmed, probable, or suspected cases in Guinea, Liberia, and Sierra Leone, including 11,310 reported deaths (as of April 13, 2016) [2].

The WHO declared the end of Ebola transmission in Guinea on 29 December 2015, in Liberia on 14 January 2016, and in Sierra Leone on 17 March 2016. However, the development of a durable and effective Ebola vaccine is a priority, both to eliminate the remnants of the outbreak and to prevent and control future epidemics.

The first wave of Ebola vaccine development, beginning soon after the discovery of the virus, focused on attempts to inactivate the virus. Since then, preclinical development of a variety of different platforms including DNA vaccines, recombinant viral vectors, recombinant proteins, subunit proteins and virus-like particles (VLPs) have been progressed.

In most cases, a replicating viral infection is very effective at eliciting robust immune responses in a host that may last for several years. This is in contrast to many recombinant antigens that are delivered either as subunit DNA plasmids or proteins; although these are considered to be reasonably safe (dependent on the adjuvant), they have frequently suffered from poor immunogenicity.

MOLECULAR BIOLOGIST

Ebola virus disease

Author: Beeching et al. (2014)
DOI: 10.1136/bmj.g7348

The virus genome consists of a single 19 kb strand of negative sense RNA with seven viral genes that are transcribed by the viral RNA dependent RNA polymerase present in the virion. The single strand of RNA is covered by helically arranged viral nucleoproteins NP and VP30, which are linked by matrix proteins VP24 and VP4 to the lipid bilayer that coats the virion.[21]
Tissue invasion occurs through infected fluid coming into contact with breaks in the mucosa or skin. This can occur with animal to human or human to human transmission. Monocytes, macrophages, and dendritic cells are the preferred replication sites for filoviruses on initial infection. Infected cells migrate to the regional lymph nodes, liver, and spleen, thereby disseminating the infection. Ebola virus has a wide cell tropism and can infect a variety of cell types.[8, 21] It also has the remarkable ability to modulate the expression of genes involved in the host immune response, causing lymphocyte apoptosis and attenuation of the protective effects of interferon.[22, 23]

The host immune response is crucial and dictates the outcome of infection. Progression to severe disease occurs when the virus triggers expression of a host of pro-inflammatory cytokines, including interferons; interleukins (ILs) such as IL-2, IL- 6, IL-8, and IL-10; interferon inducible protein; and tumour necrosis factor α (TNF-α).[8-24] This causes endothelial activation and reduced vascular integrity, release of tissue factor (with associated onset of coagulopathy), and increased nitric oxide levels (with associated hypotension).[25]

The main confirmatory test for Ebola virus infection is a positive Ebola RT-PCR.[48] This test should be ordered in all patients with suspected Ebola infection while the patient is isolated. The results of RT-PCR are available 24-48 hours before those of enzyme linked immunosorbent assay (ELISA) testing. In Western settings, Ebola RT-PCR may be available only in regional or national reference laboratories that have a high level of biosafety precautions (category 4 facilities).[8] In epidemic settings and some European countries, category 4 laboratories are set up locally, and RT-PCR is available four hours after the sample has arrived. Viral RNA can be detected in the patient's blood by RT-PCR from day 3 to days 6-17 of symptoms. A positive result implies that the patient is potentially infectious, particularly if there is active diarrhoea, vomiting, or bleeding. If negative, the test should be repeated within 48 hours because viral load can be low and undetectable early in the illness. Negative tests should also be repeated to rule out the diagnosis (or confirm resolution of infection) if there is a strong suspicion of Ebola.[31] Higher viral load correlates with adverse outcome.[5-49]

Functional CD8 T Cell Responses in Lethal Ebola Virus Infection

Author: Bradfute et al. (2008)
DOI: 10.4049/jimmunol.180.6.4058

The activation status of lymphocyte subsets during the course of EBOV infection have not been functionally studied; for example, the question of whether lymphocytes undergo apoptosis after partial activation, which could occur in the presence of incompletely activated dendritic cells, remains unanswered. To address these issues, a lethal mouse model of EBOV virus infection, in which death occurs on or around day 7 postinfection, was used. Lymphocyte responses to EBOV in mice are similar to those seen in nonhuman primates and humans, including classical lymphocyte apoptosis [9, 10]. Serial sampling experiments were performed, spleen and blood were harvested daily after infection, and lymphocytes were analyzed for activation markers. There was an increase in the percentage of CD4 and CD8 T cells expressing high levels of CD44, an activation and maturation marker, toward the end of lethal EBOV infection. Increased expression of CD44 correlated with increased lymphocyte numbers in the blood and previously reported lymphoblast presence in the spleen [9], which are indicative of lymphocyte activation and proliferation. These data led to a hypothesis that a functional lymphocyte response was being generated in lethal EBOV infection.

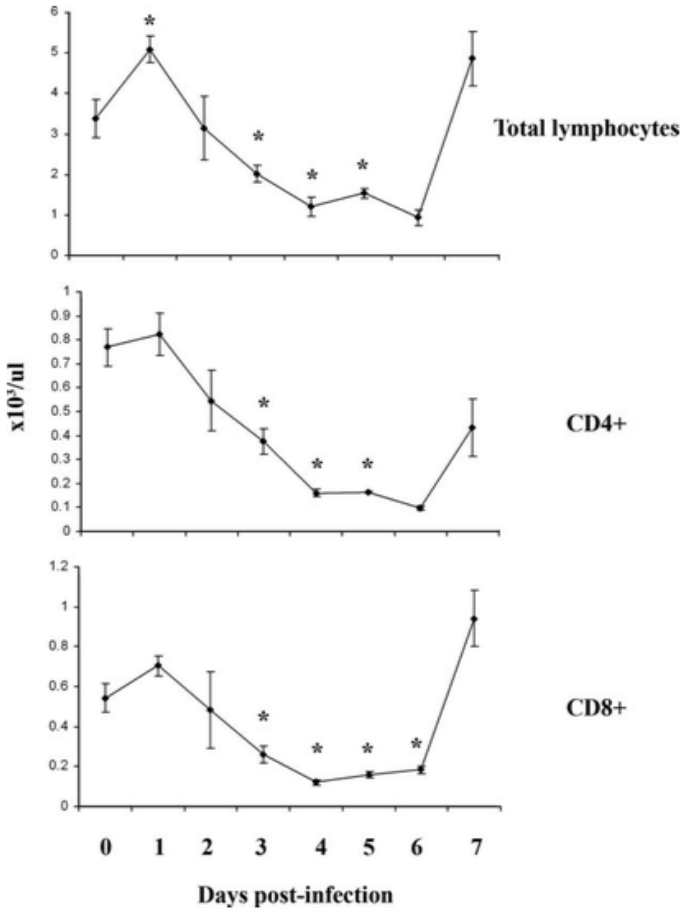


FIGURE 1. Lymphocyte numbers in blood during the course of EBOV infection in mice. C57BL/6 mice were infected with 1000 PFU of mouse-adapted EBOV, and blood and spleen were harvested daily and analyzed. Lymphocyte numbers in the blood diminish during the course of EBOV infection but rebound shortly before death. CD4 and CD8 cell numbers show the same trend, although the recovery is more prominent in CD8 cells.

There are several possible explanations as to why this immune response is insufficient for survival in this model. First, the response may be too late to control the rapidly replicating virus, which can reach serum titers of up to 108 PFU/ml [9]. Although the development of the CD8 response described here is similar to what would be expected from a successful immune response, the speed at which disease occurs may outpace the response. Similarly, the number of CD8 T cells that are generated could be too low to control high viral titers but are sufficient upon

transfer to newly infected animals to control the disease. Secondly, the immune response could be dampened by altered cytokine production in the day 7 animals and are more functional or effective after transfer to a newly infected animal. Third, the damage done by infection could be too severe to overcome at the time the adaptive immune system responds.

Ebola virus: from discovery to vaccine
Author: Feldmann et al. (2003)

A scientific breakthrough in the field was made with the development of the infectious clone for EBOV[9,10] (**Figure 2**). This success has to be attributed to the knowledge that was gained from work done with artificial mini-genome systems[11,12]. The infectious clone system was used to create an editing site mutant that led to the overexpression of transmembrane glycoprotein (GP) and the loss of expression of soluble glycoprotein (sGP)[9]. The recombinant virus had an increased cytopathogenic effect, but reduced virus production, indicating that RNA editing is required to reduce the cytotoxicity of transmembrane GP[9,13]. A slightly different, and independently established, system was used to study the role of proteolytic cleavage for the activation of transmembrane GP[10,14]. A recombinant virus that expresses non-cleaved GP molecules on the surface was isolated and shown to be infectious in tissue culture, indicating that proteolytic cleavage is not a requirement for infectivity[10]. The infectious clone system will be instrumental for future studies on protein function, genome transcription and replication, pathogenesis and the development of therapeutic and prophylactic interventions.

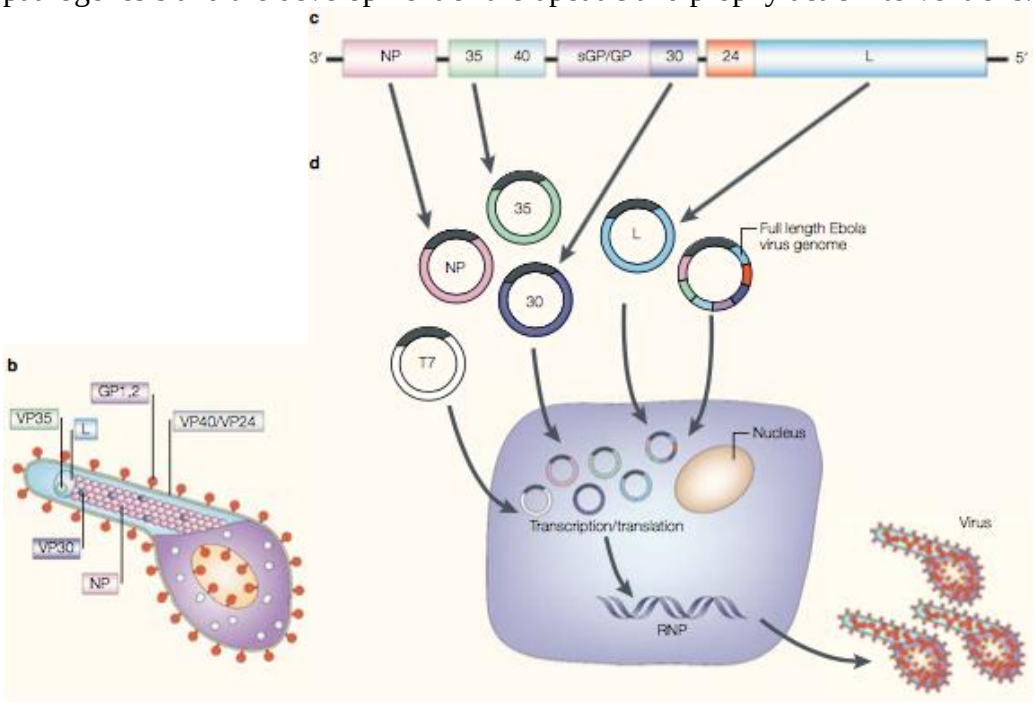


Figure 2 | **Filovirus particles. b** | Schematic illustration of a particle. Four proteins are involved in the formation of the ribonucleoprotein complex (RNP): polymerase or large (L) protein, nucleoprotein (NP), virion structural protein 30 (VP30) and VP35. The glycoprotein (GP) is a type I transmembrane protein and is anchored with the carboxy-terminal part in the virion membrane. Homotrimers of GP form the spikes on the surface of the virion. VP40 and VP24 are

membrane-associated proteins. **c** | A schematic illustration of the Ebola virus (EBOV) genome. The genome consists of a single, negative-stranded, linear RNA molecule. **d** | Generation of EBOV particles from cloned complementary DNA. The system is based on the co-transfection of six different plasmids: five expression plasmids under the control of the chicken β-actin promoter, encoding the four virus proteins that are required for transcription and replication (NP, VP30, VP35 and L) of the EBOV genome and the bacteriophage T7 polymerase, and a plasmid under the transcriptional control of the T7 promoter for transcribing the full-length EBOV genome.

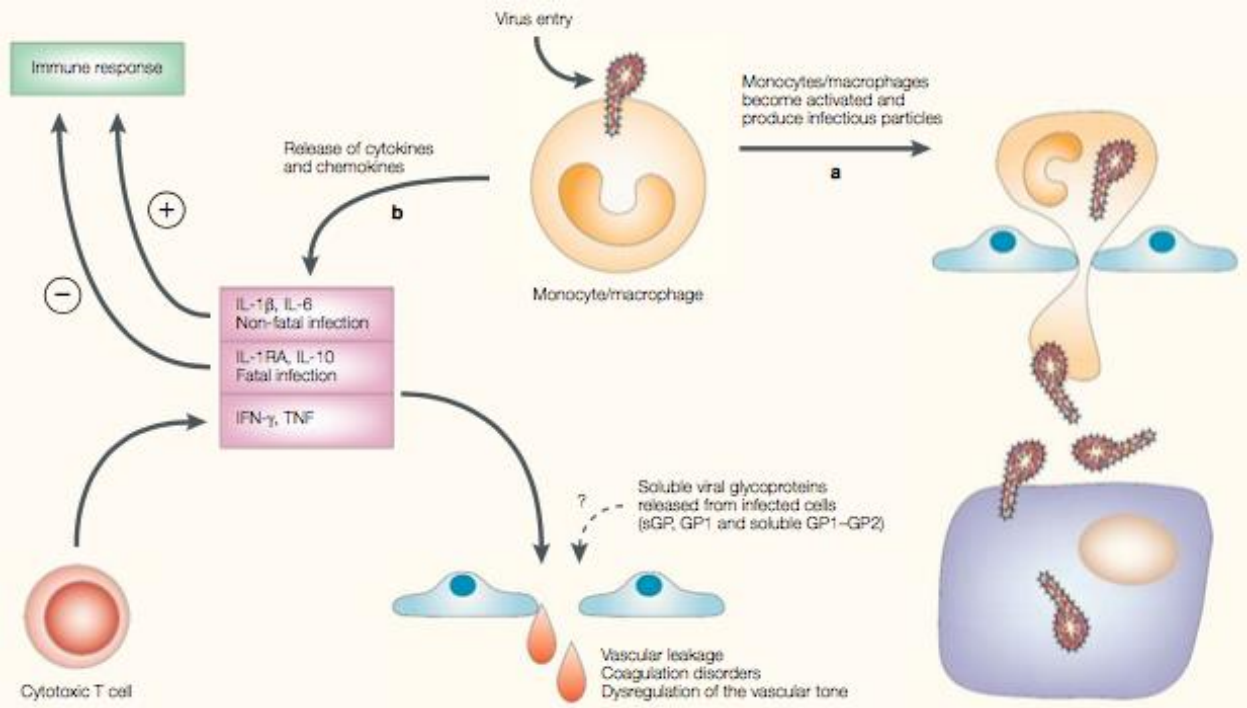


Figure 3 | **Pathogenesis model.** The concept of the pathogenesis of filovirus haemorrhagic fever is illustrated. Viruses that enter the body through lymphatic and/or blood vessels get direct access to sessile monocytes/macrophages that become activated independently of virus replication. **a** | Infected cells might extravasate into tissues to infect other cells, such as hepatocytes, and induce focal necrosis. **b** | The production of cytokines by monocytes/macrophages can either promote or inhibit the immune response. Pro-inflammatory cytokines, such as interferon-γ (IFN-γ; additionally produced by cytotoxic T cells22) and tumour-necrosis factor (TNF), can induce the activation of endothelial cells and increase vascular leakage. Direct infection of endothelial cells occurs, but probably has a minor role in the pathogenesis.

EVOLUTIONARY BIOLOGIST

Emergence of Zaire Ebola Virus Disease in Guinea
Author: Baize et al. (2014)
DOI: 10.1056/NEJMoa1404505

The EBOV in samples obtained from three patients was completely sequenced with the use of conventional Sanger techniques (GenBank accession numbers: KJ660346, KJ660347, and KJ660348). The three sequences, each 18,959 nucleotides in length, were identical, with the exception of a few polymorphisms at positions 2124 (G→A, NP552 glycine→glutamic acid), 2185 (A→G, synonymous), 6909 (A→T, sGP291 arginine→tryptophan), 9923 (T→C, synonymous), 13856 (A→G, L759 aspartic acid→glycine), and 15660 (T→C, synonymous). The Guinean EBOV strain showed 97% identity to EBOV strains from the Democratic Republic of Congo and Gabon. Phylogenetic analysis of the full-length sequences by means of Bayesian and maximum-likelihood methods revealed a separate, basal position of the Guinean EBOV within the EBOV clade (**Figure 3**).

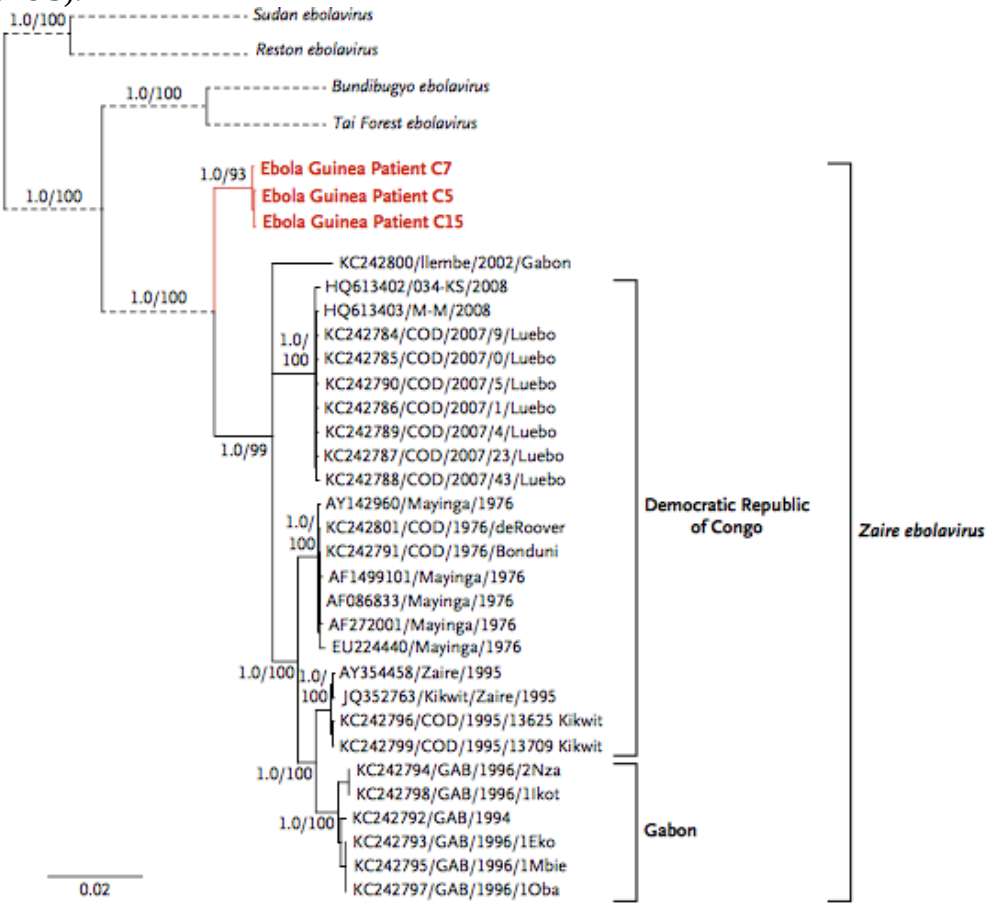


Figure 3. Phylogenetic Analysis of the Ebolavirus Genus, Including the EBOV Strains from Guinea. The phylogenetic tree was inferred with the use of the Bayesian Markov Chain Monte Carlo method. Bayesian posterior probabilities and bootstrap percentages (1000 replicates of the maximum-likelihood tree) are shown on the branches. The GenBank accession number, strain designation, country of origin, and year of isolation are indicated on the EBOV branches.

Phylogenetic analysis of the full-length sequences established a separate clade for the Guinean EBOV strain in a sister relationship with other known EBOV strains. This suggests that the EBOV strain from Guinea has evolved in parallel with the strains from the Democratic Republic of Congo and Gabon from a recent ancestor and has not been introduced from the latter countries into Guinea. However, the determination of both the timing of the introduction of the virus into Guinea and its phylogenetic origin also depend on our understanding of the evolutionary rate of EBOV in nature

The ecology of Ebola virus
Author: Groseth et al. (2007)
DOI: 10.1016/j.tim.2007.08.001

Scientists have long hypothesized that EBOV must persist in the endemic areas in a classical zoonotic reservoir in which it does not cause disease, or does so only infrequently. Given that apes succumb to EBOV infection following a severe disease similar to that in humans, these species are not considered a classical reservoir species. Thus, following the discovery of EBOV in 1976, and again after the 1994 case in Ivory Coast and the 1995 outbreak in DRC, intensive efforts were made to identify the natural reservoir; however, neither potential hosts nor arthropod vectors were identified [22–25]. Later a report by Morvan et al. [26] indicated detection of Zaire ebolavirus (ZEBOV) RNA in rodents (Muridae and Soricidae) captured in the Central African Republic (CAR), and proposed mice, rats and shrews as possible reservoir species.

Figure 3. Mechanisms of filovirus transmission in nature. Known and hypothesized mechanisms of transmission between reservoir, potential amplifying hosts and susceptible hosts, including humans, are summarized. Arrow colors indicate whether the transmission follows a known (green arrow), suspected (blue arrow) or hypothetical mechanism (purple arrow).

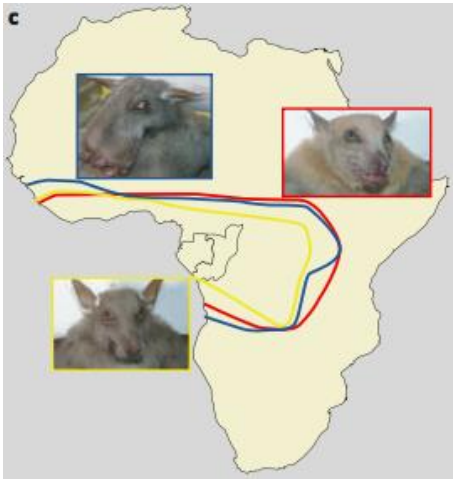
There are currently two theories to explain the transmission of filoviruses among susceptible hosts in nature. The first suggests that, as with many classical viral zoonotic pathogens, the virus has been maintained in endemic regions within a reservoir host and its episodic emergence has

occurred owing to infrequent contact between the reservoir(s) and humans or nonhuman primates. However, an alternative mechanism of spread has been proposed, whereby the virus has been more recently introduced into susceptible populations and spread in a wave-like fashion to each outbreak site through an undefined reservoir host (**Figure 3**).

Fruit bats as reservoirs of Ebola virus
Author: Leroy et al. (2005)
DOI: 10.1038/438575a

To identify the viral reservoir, we undertook three trapping expeditions in areas close to infected gorilla and chimpanzee carcasses, just after their discovery. In total, 1,030 animals were captured, including 679 bats, 222 birds and 129 small terrestrial vertebrates, and were tested for evidence of infection by Ebola virus. Of the infected animals identified during these field collections, immunoglobulin G (IgG) specific for Ebola virus was detected in serum from three different bat species (4 of 17 *Hypsignathus monstrosus*, 8 of 117 *Epomops franqueti* and 4 of 58 *Myonycteris torquata*). Two of the principal organs targeted by Ebola virus are the liver and spleen[4]. Viral nucleotide sequences were detected in these organs in other bats from the same populations (4 of 21, 5 of 117 and 4 of 141, respectively). No viral RNA was detected in kidney, heart or lung in these animals after amplification by polymerase chain reaction (PCR) and no viral nucleotide sequences were revealed in any of the other animal species tested.

Each of the three bat species has a broad geographical range that includes regions of Africa where human Ebola outbreaks occur[5] (**Figure 1C**). Our findings support results of previous investigations that identify bats as candidate reservoirs for Ebola and Marburg viruses[1,6], and as reservoirs for the virus families *Paramyxoviridae* and *Rhabdoviridae*[7–9], which are genetically related to Ebola.

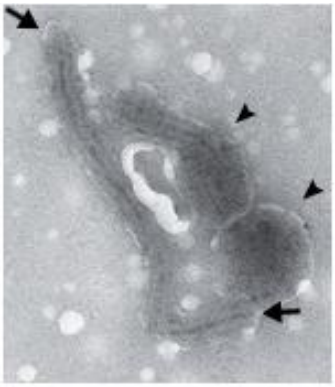


Mortality among great apes from Ebola infection can increase during the dry seasons[3] when fruit is scarce in the forest - conditions that foster contact between animals as they compete for food. Immune function in bats also changes during these periods[10], for example as a result of food scarcity or pregnancy, which would favour viral replication and - aided by aggressive interactions - increase infection among great apes. These factors may contribute to the episodic nature of Ebola outbreaks.

EBOLA

STATISTICIAN

Emergence of Zaire Ebola Virus Disease in Guinea
Author: Baize et al. (2014)
DOI: 10.1056/NEJMoa1404505



EBOLA

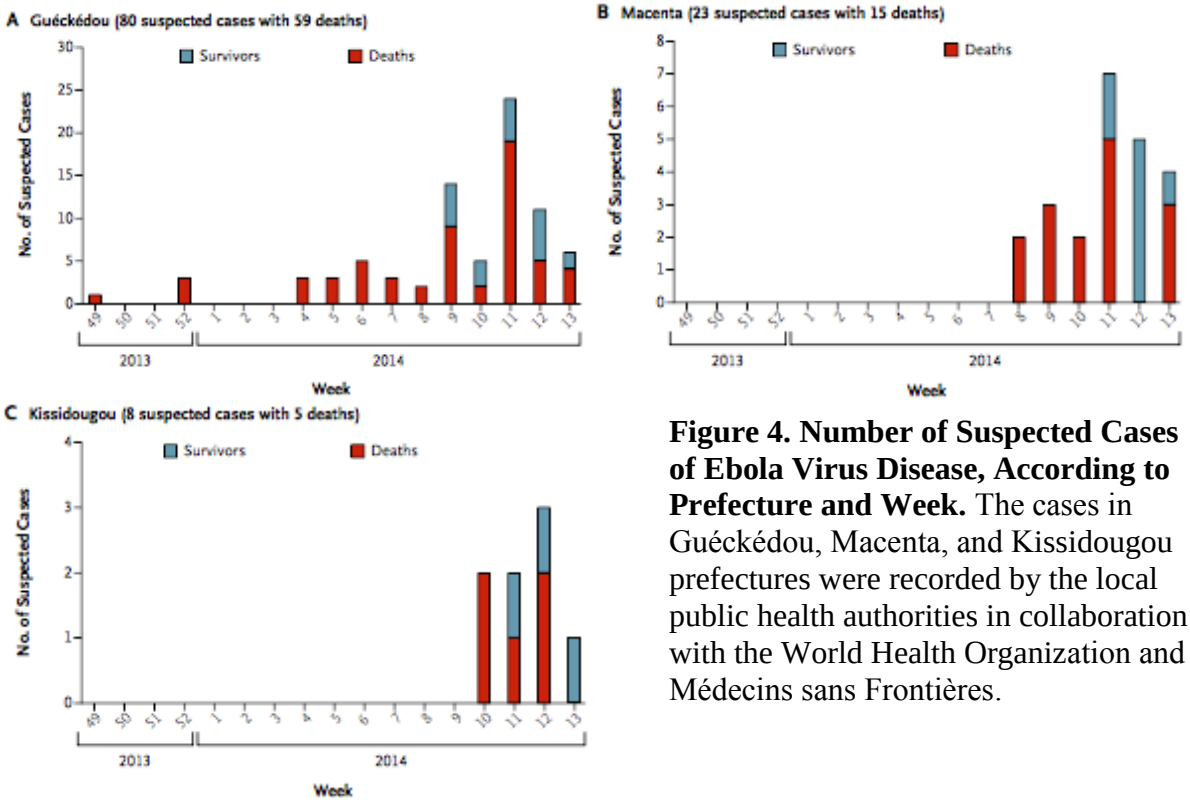


Figure 4. Number of Suspected Cases of Ebola Virus Disease, According to Prefecture and Week. The cases in Guéckédou, Macenta, and Kissidougou prefectures were recorded by the local public health authorities in collaboration with the World Health Organization and Médecins sans Frontières.

The clinical picture of the initial cases was predominantly fever, vomiting, and severe diarrhea. Hemorrhage was not documented for most of the patients with confirmed disease at the time of sampling but may have developed later in the course of the disease. The term Ebola virus disease (rather than the earlier term Ebola hemorrhagic fever [EHF]) takes into account that hemorrhage is not seen in all patients[15] and may help clinicians and public health officials in the early recognition of the disease. The case fatality rate was 86% among the early confirmed cases and 71% among clinically suspected cases, which is consistent with the case fatality rates observed in previous EBOV outbreaks.[15-17]

Figure 1. Map of Guinea Showing Initial Locations of the Outbreak of Ebola Virus Disease. The area of the outbreak is highlighted in red. The main road between the outbreak area and Conakry, the capital of Guinea, is also shown. The map was modified from a United Nations map.

Efficacy and effectiveness of an rVSV-vectored vaccine in preventing Ebola virus disease: final results from the Guinea ring vaccination, open-label, cluster-randomised trial (Ebola Ça Suffit!)

Author: Henao-Restrepo et al. (2016)
DOI: 10.1016/ S0140-6736(16)32621-6

The Guinea ring vaccination trial was a cluster-randomised controlled trial designed to assess the effect of one dose of the candidate vaccine in protecting against laboratory confirmed Ebola virus disease. Ebola virus spread across many geographical areas of Guinea, mainly through familial and social networks and funeral exposures.[19] After confirmation of a case of Ebola virus disease (index case), we enumerated and randomised clusters (called rings) of epidemiologically linked people.[20] The ring vaccination design ensured that the study was undertaken in pockets of high incidence of Ebola virus disease despite the declining epidemic and an overall low attack rate (ie, the total number of cases of Ebola virus disease in the three worst affected countries divided by the estimated total population of these countries; estimated here as about 0·13%).

Contacts and contacts of contacts of individuals with Ebola virus disease were enumerated into clusters (and the information stored on a list) and these clusters were cluster-randomised (1:1) to either immediate vaccination or delayed vaccination (21 days later) of all eligible individuals.[20] The teams who defined the clusters were different from the team who took informed consent or did the vaccinations. Randomisation took place only after the list enumerating all the contacts and contacts of contacts of a cluster was closed. An independent statistician not otherwise involved in the trial generated the allocation sequence, and Ebola response teams and laboratory workers were unaware of the allocation of clusters.

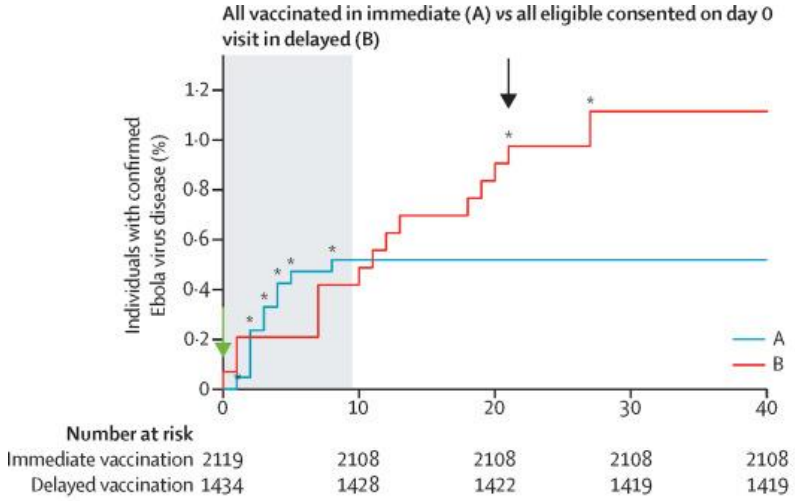


***Figure 3*: Kaplan-Meier plots for confirmed cases of Ebola virus disease in different study populations**

Arrows show time of vaccination (at day 0 or day 21); the plus signs denote cases among non-eligible children and the stars denote cases among vaccinated individuals; the shaded area denotes the *a priori* (based on deduction rather than observation) defined lag time of 0–9 days. **Blue line:** A few cases occurred within the first few days after vaccination, shortly thereafter there were no further infections. **Red line:** Steady increase of EBV with a reduction once the control arm was provided vaccine 21 days later.

The ecology of Ebola virus
Author: Groseth et al. (2007)
DOI: 10.1016/j.tim.2007.08.001

Interestingly, with the exception of the two outbreaks in 1976 and 1995 in DRC, all reported Zaire ebola virus (ZEBOV) outbreaks seem to be associated with the region near the border of Gabon and the Republic of the Congo (RC), the latest of which took place in 2005 at Etoumbi and Mbomo in RC [18]. By contrast, all Sudan ebolavirus (SEBOV) outbreaks reported to date have occurred either in Sudan or in neighboring Uganda, and only two isolated human cases f Ivory Coast ebolavirus (ICEBOV) have been reported in Ivory Coast and Liberia [9,19]. To date, there have been a total of 1860 reported cases of ebola hemorrhagic fever (EHF), resulting in 1296 deaths.

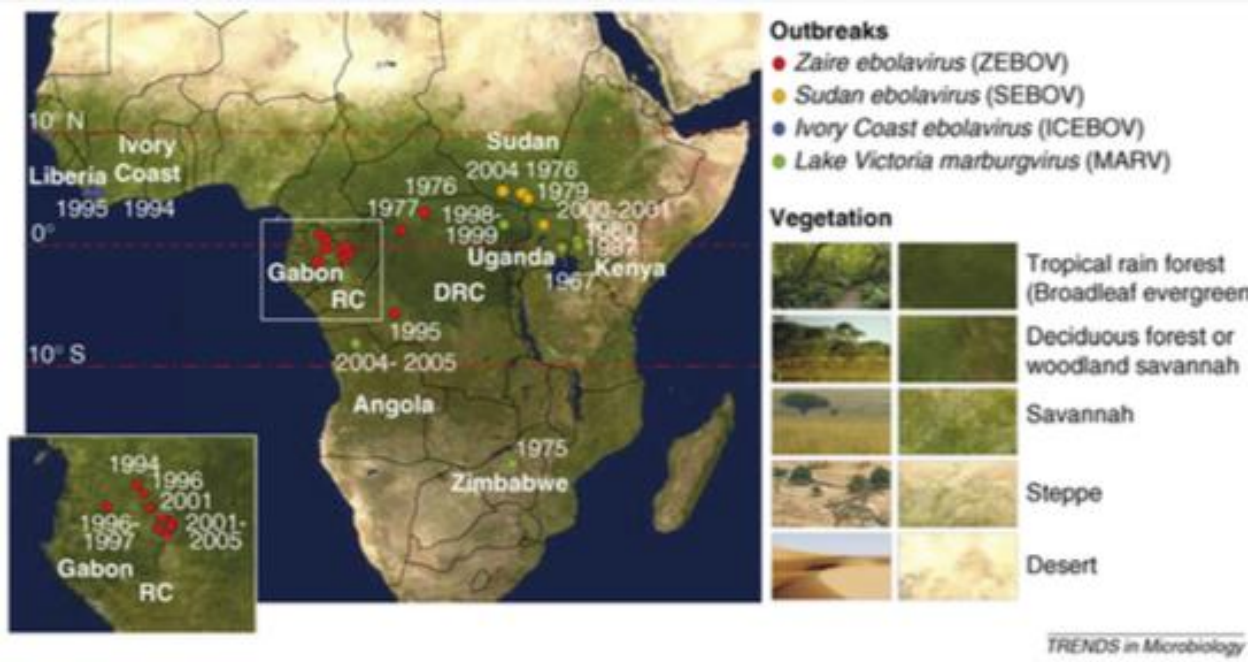


Figure 1. Spatial distribution of human filovirus outbreaks in relation to geographical conditions. The political boundaries and the names of the countries where Ebola virus or Marburg virus outbreaks have been reported are indicated, as are the relevant lines of latitude. The site of each Ebola virus or Marburg virus outbreak is also indicated, as well as the year in which the outbreak occurred. Color patterns in the satellite image are shown at the side, correlated to the ecological conditions they represent.

Ebola Virus Disease in West Africa — The First 9 Months of the Epidemic and Forward Projections
Author: WHO Ebola Response Team (2014)
DOI: 10.1056/NEJMoa1411100

EBOLA

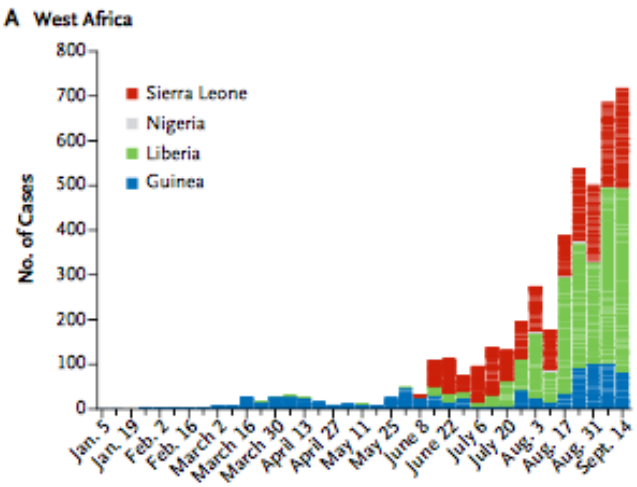


Figure 2. Weekly Incidence of Confirmed, Probable, and Suspected Ebola Virus Disease (EVD) Cases. Shown is the weekly incidence of confirmed, probable, and suspected EVD cases, according to actual or inferred week of symptom onset. A suspected case is illness in any person, alive or dead, who has (or had) sudden onset of high fever and had contact with a person with a suspected, probable, or confirmed Ebola case or with a dead or sick animal; any person with sudden onset of high fever and at least three of the following symptoms: headache, vomiting, anorexia or loss of appetite, diarrhea, lethargy, stomach pain, aching muscles or joints, difficulty swallowing, breathing difficulties, or hiccupping; or any person who had unexplained bleeding or who died suddenly from an unexplained cause. A probable case is illness in any person suspected to have EVD who was evaluated by a clinician or any person who died from suspected Ebola and had an epidemiologic link to a person with a confirmed case but was not tested and did not have laboratory confirmation of the disease. A probable or suspected case was classified as confirmed when a sample from the person was positive for Ebola virus in laboratory testing.

IMMUNOLOGIST

Ebola virus disease

Author: Beeching et al. (2014)
DOI: 10.1136/bmj.g7348

Transmission occurs by close contact with body fluids of infected patients. The incubation period after infection is usually 5-9 days, with a range of 1-21 days in 95% or more of patients,[2, 3] and patients are not considered infectious until they develop symptoms. The initial presentation is non-specific, which makes early clinical diagnosis difficult. Human infection carries a high case fatality rate depending on the species of Ebola virus and quality of supportive care available.[4, 5] Ebola virus infection (formerly Ebola haemorrhagic fever) is part of a group of diseases known as viral haemorrhagic fevers.[6]

The virus is thought to be initially acquired by exposure to body fluids or tissue from infected animals, such as bats and non-human primates; however, the natural reservoir and mode of transmission to humans has not been confirmed.[7, 8] Laboratory testing of reservoir competence shows that successful infection is possible in bats and rodents, but not in plants or arthropods.[9-12] Animal to human transmission may occur during hunting and consumption of the reservoir species or infected non-human primates. The practice of butchering or eating bush meat or food contaminated with bat faeces (three species of tree roosting bats have been implicated as a reservoir) is also thought to contribute.

Human to human transmission occurs through contact with body fluids from infected patients.[13] In early epidemics, the re-use of non-sterile injections was responsible for many healthcare associated transmissions.[14] However, although this remains a risk, most cases result from close physical contact or contact with body fluids (such as sweat, blood, faeces, vomit, saliva, genital secretions, urine, and breast milk) of infected patients. In a study of viral shedding in various body fluids, Ebola virus was isolated from saliva, breast milk, stool, tears, and semen up to 40 days after the onset of illness,[15-17] confirming the possibility of delayed sexual transmission. Virus may be found in urine during recovery, and the duration of this phenomenon needs further study.[18] Infection through inhalation is possible in non-human primates, but there is no evidence for airborne transmission in humans.[19]

Outside endemic areas, Ebola virus infection is rare and is usually imported.[20] Travellers from affected areas, and laboratory scientists and others working with potentially infected materials and animals, are at high risk.

The ecology of Ebola virus

Author: Groseth et al. (2007)
DOI: 10.1016/j.tim.2007.08.001

To date, four species of Ebola virus (EBOV) have been described, of which three, Zaire ebolavirus (ZEBOV), Sudan ebolavirus (SEBOV) and Ivory Coast ebolavirus (ICEBOV), co-circulate in Africa [1–3]. Of these species, ZEBOV and SEBOV have proved highly pathogenic

for both human and nonhuman primates, causing viral hemorrhagic fever (VHF; see Glossary) with case fatality rates of up to 90% [4,5], for which no approved therapeutics or vaccines are currently available [6]. Following an incubation period typically of 4–10 days [7], victims rapidly develop a fever of >38.5 ºC combined with other relatively non-specific early signs [8–10]. Subsequently, 50% of patients develop a maculopapular rash on the trunk and shoulders, and the majority of patients show some signs of impaired coagulation; however, massive bleeding is rare and is mainly restricted to the gastrointestinal tract [11]. In fatal cases, death occurs between 6 and 16 days after the onset of symptoms [2] as a result of multi-organ failure and shock [7,8]. Owing to the similarities with other conditions found in these regions, the diagnosis is easily missed early in the disease course. It is often only after failure to respond to anti-malarial and/or antibiotic treatment, and also often only after health care providers fall victim to these diseases, that cases are recognized.

Ebola Virus Disease in West Africa — The First 9 Months of the Epidemic and Forward Projections
Author: WHO Ebola Response Team (2014)
DOI: 10.1056/NEJMoa1411100

The most common symptoms reported between symptom onset and case detection included fever (87.1%), fatigue (76.4%), loss of appetite (64.5%), vomiting (67.6%), diarrhea (65.6%), headache (53.4%), and abdominal pain (44.3%). Specific hemorrhagic symptoms were rarely reported (in <1% to 5.7% of patients). “Unexplained bleeding,” however, was reported in 18.0% of cases.

Immunopathology of highly virulent pathogens: insights from Ebola virus
Author: Zampieri et al. (2007)
DOI: 10.1038/ni1519

The uncontrolled viral replication of Ebola virus is central to its pathogenesis, both because of its cytopathic effects and because it induces prominent dysregulation of the host immune response. Virally induced immune system impairment occurs through a variety of mechanisms. Studies in nonhuman primates as well as guinea pigs raise the possibility that monocytes, macrophages and dendritic cells are early and preferred sites of viral replication[8,9], though it remains possible that virus is present on these cells through binding to lectin receptors rather than active replication *in vivo.* It has been suggested that these cells act as vehicles for the transport of virus through the lymphatics[10]. Further viral replication ensues, followed by systemic spread to other organs and tissues (**Figure 1B**). Although virus is observed in the reticuloendothelial system, little inflammation is seen within the lymphatics or in infected tissues during the course of the infection.

Infection of monocytes and macrophages leads to the release of pro- inflammatory cytokines and chemokines, including tumor necrosis factor, interleukin-1β, macrophage inflammatory protein-1α and reactive oxygen and nitrogen species[11,12]. The expression of these mediators is likely to attract more monocytes and macrophages to the sites of infection and may also attract

neutrophils. Although recent data suggests that they are not productively infected, human neutrophils reacted with filovirus *in vitro* show rapid activation of triggering receptor expressed on myeloid cells-1 (TREM-1)[13]; this results in the release of further inflammatory cytokines and chemokines that contribute to vasodilation and increased vascular permeability. In addition, infected monocytes and macrophages express cell surface tissue factor, which may be involved in the development of coagulopathies[14]. After productive infection, macrophages undergo cell lysis and apoptosis in large numbers[15]; thus, activated monocytes and macrophages do not seem to deter viral spread. Rather, they may contribute to dissemination by supporting viral replication or by transporting virus bound to cell surface lectin binding proteins within the lymphatic system. And like neutrophils, monocytes and macrophages may also secrete soluble factors that exacerbate pathogenic manifestations of the disease[13].

Like monocytes and macrophages, immature dendritic cells (DCs) are 'targets' of Ebola virus, either by means of attachment of viral particles through interactions with DC-expressed C-type lectin DC-SIGN or by means of infection through interaction with other DC-expressed cell-surface receptors (**Figure 1C**). Dendritic cells are among the most effective antigen-presenting cells of the immune system, and they secrete critical interleukins and cytokines that provide a critical link between innate and adaptive immune responses to many pathogens; DCs infected with Ebola virus are severely compromised in these critical functions.

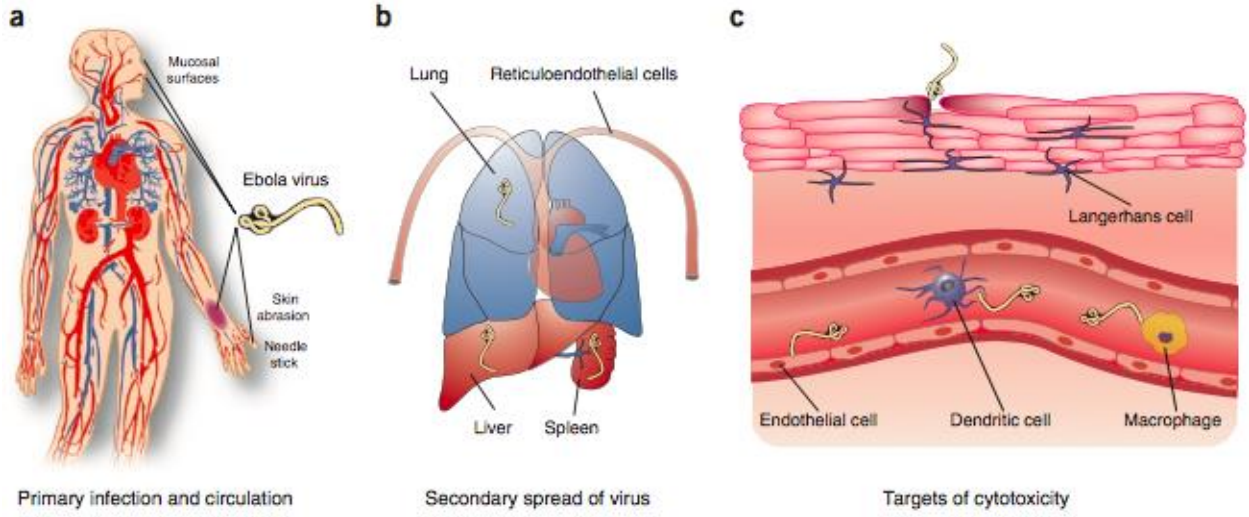


Figure 1. Infection, spread and target cell destruction by Ebola virus. (**A**) Ebola virus (yellow) infects subjects through contact with body fluid or secretions from an infected patient and is distributed through the circulation. Entry can occur through abrasions in the skin during patient care, burial rituals and possibly contact with infected bushmeat, or across mucosal surfaces. Accidental needle stick is the primary route of occupational exposure. (**B**) Early targets of replication are reticuloendothelial cells, with high replication in several cell types within the lungs, liver and spleen. (**C**) Dendritic cells, macrophages and endothelium appear to be susceptible to cytopathic effects of Ebola virus gene products *in vitro* and possibly *in vivo* through disruption of cellular signaling pathways affected by virus binding, phagocytic uptake or both. Indirect damage may also be inflicted by circulating factors such as tumor necrosis factor and nitric oxide.

EBOLA

Surprisingly, patients who succumb to Ebola virus infection show little evidence of an activated adaptive immune response. Adaptive immunity is severely compromised not only because of a lack of functional DCs and other important antigen-presenting cells, but also because lymphocytes undergo massive apoptosis in infected humans and nonhuman primates[15,30,31]. Although lymphocytes are not targets of the virus, substantial numbers - with the exception of B cells - undergo apoptosis during the illness[32]; as a result, numbers of CD4+ and CD8+ T cells are substantially reduced in fatal human and nonhuman primate infections before death[30,31,33]. Lymphocyte apoptosis is also a common manifestation of other viral hemorrhagic fevers and is frequently observed during septic shock[34.]

POTATO BLIGHT

HISTORICAL CONTEXT

Tracking historic migrations of the Irish potato famine pathogen, *Phytophthora infestans*
Author: Ristaino (2002)
Late blight caused by the plant pathogen *Phytophthora infestans* is a devastating disease of potato and tomato in the U.S. and worldwide (Fig. 1) [1]. The pathogen causes a destructive foliar blight and also infects potato tubers and tomato fruit under cool, moist conditions (Fig. 1). The pathogen can be transported long distances in infected plant materials.

Epidemics caused by *P. infestans* in 1845 led to the Irish potato famine and the mass emigration and death of millions of people in Ireland. The first record of the occurrence of late blight in the United States was in 1843 around the port of Philadelphia [2]. Epidemics of late blight subsequently spread to a five-state area and Canada over the next 2 years. In 1845, late blight epidemics also occurred in Belgium, Holland, Germany, Switzerland, France, Italy, England, Ireland and Scotland. For the people in Ireland, who subsisted on the potato as a main food source, the late blight epidemics were devastating [1]. An average male consumed 12 pounds of potatoes per day. In a 5-year period, the population in Ireland decreased dramatically as over 1.5 million people died from starvation and disease and an equal number emi- grated from Ireland [3].

Epidemics of potato late blight occurred before the germ theory had been clearly elucidated. Some believed weather was responsible for the malady on potato [2]. Others blamed the devil or bad soil. In a pamphlet written on late blight in Scotland, it was said, "It is certain that a fungus appears in the leaves, stems, and tubers of the plants which have been attacked, but it is uncertain how far the fungus is the cause or the consequence of the disease — how far it is to be considered as a parasite upon the living potato, or as a mere devourer of its dying parts" [4]. The painstaking work of J. Teschemacher in the United States, M.J. Berkeley in Great Britain, Montagne in France, and later DeBary in Germany, clearly elucidated that a fungus-like organism was responsible for the disease [5,6]. Late blight epidemics appeared in Europe before Louis Pasteur's pioneering work on the germ theory of disease. Research by early mycologists who studied the late blight pathogen, was some of the first to document that fungi were capable of causing plant disease and laid the groundwork for the discipline of plant pathology [5,6].

Late blight has become a reemerging disease worldwide in recent years, more than 150 years after the great famine. The disease has reached epidemic proportions in North America, Russia, and Europe due to the development of resistance to phenylamide fungicides in populations of the pathogen and the widespread occurrence of new genotypes [7–9]. The disease has been responsible for the extensive use of fungicides on potato, and in many areas of the world the crop cannot be grown without their frequent application.

"What a Painfully Interesting Subject": Charles Darwin's Studies of Potato Late Blight
Author: Ristaino & Pfister (2016)
DOI: 10.1093/biosci/biw114

Potato late blight a remerging plant disease threat

POTATO BLIGHT

Potato late blight caused by *Phytophthora infestans*, a member of the Oomycota, was responsible for the plant disease that led to famine in Ireland of 1845 (Berkeley 1846). The pathogen is still wreaking havoc on potatoes and tomatoes in the United States and many areas of the world and is considered a threat to global food security (Hu et al. 2012, Fry et al. 2015). To control late blight, fungicides are applied at a higher rate than on any other food crop at global costs exceeding $1 billion.

Phytophthora infestans is considered a serious threat to food security and is a reemerging disease for several reasons. The pathogen has a polycyclic life cycle, and aerial sporangia can be dispersed over distances of many kilometers and therefore easily spread (Fry et al. 2015). The pathogen can also be transported in infected tubers, tomato fruit, and infected transplants (Hu et al. 2012). Fungicide-resistant strains of the pathogen emerged shortly after the release of the phenyamide fungicide metalaxyl in the 1980s (Fry et al. 2015). In addition, the monoculture of highly susceptible potato varieties in the United States has exacerbated disease. R-gene based resistance in potato has been deployed and has been unsuccessful; the pathogen genome is plastic, and a suite of effector proteins have evolved to evade host-plant R-gene- mediated resistance derived from *Solanum demissum*.

Potential effects of diurnal temperature oscillations on potato late blight under climate change: Results from experiments and simulation modeling
Author: Shakya (2014)

Potato, Late Blight and its Causal Agent Phytophthora infestans
Potato (Solanum tuberosum L.) is the third most important food crop after wheat and rice with global production of more than 300 million metric tons. It is cultivated under temperate, subtropical and tropical conditions. More than half (52%) of the potato production is in 18 developing countries. The cultivated potato originated in the Andes in Peru around Lake Titicaca, where a wide variety of primitive cultivars and wild species-relatives still exist (Spooner et al. 2005).

Late blight is one of the most important and economic diseases of the potato crop, and have the potential of destroying a whole potato field in a week under favorable conditions. The disease is caused by an oomycete, *Phytophthora infestans* (Mont.) de Bary. The pathogen is more closely related to brown algae and diatoms than to fungi, as evident from the molecular phylogenies (Baldauf et al. 2000; Sogin and Silberman 1998; Thines and Kamoun 2010). ... *P. infestans* can reproduce both sexually and asexually. It is a heterothallic oomycete and requires two opposite mating types (A1 and A2) and produces a thick-walled sexual resting structure called oospore. Oospores can survive from one season to another in soil … and can remain in soil for many years (Andersson et al. 1998; Lehtinen and Hannukkala 2004). The production of oospores is not very common as it requires both mating types (A1 and A2); instead, *P. infestans* commonly produces asexual spores called sporangia. A single lesion could have as many as 300,000 sporangia (Mayton et al. 2000) which are distributed through wind. Sporangia do not survive for a long time in the atmosphere (Fernández-Pavía et al. 2004; Mizubuti et al. 2000; Sunseri et al. 2002) as their survival is dependent on temperature, relative humidity and solar radiation (Minogue and Fry 1981).

POTATO BLIGHT

Late blight disease means early end for tomato crops

Author: Mabbett (2016)

Phytophthora infestans, the causal pathogen of late blight disease, made its mark on the potato crop (*Solanum tuberosum*) causing successive European, Irish and Scottish Highlands' potato famines through the 1840's. Be that as it may, the potato's close relative, the tomato (*Lycopersicon esculentum*), grown just as widely and on balance more sensitive to microbial disease, suffers equally, if not more acutely than potato does, at the hands of this particular plant pathogen.

The disease is no longer regarded as a true fungus. It is simply described as a fungus-like pathogen, positioned in a wider classification grouping called Oomycota, commonly referred to as the water moulds. However, this taxonomical re-arrangement in no way detracts from the pathogenicity of *P. infestans* and its ability to cause late blight in selected plant members of the Solanaceae. For tomato this means an early end to the crop.

One factor in favour of tomato growers faced with risks from late blight disease, is that the pathogen has a limited host range, confined to selected members of the plant family Solanaceae. For instance, neither the tobacco crop *(Nicotiana tabacum)* nor black nightshade (*Solanum nigrum*), one of the most widely spread arable weeds of the plant family Solanaceae, is susceptible to infection by *P. infestans* and therefore unaffected by late blight disease.

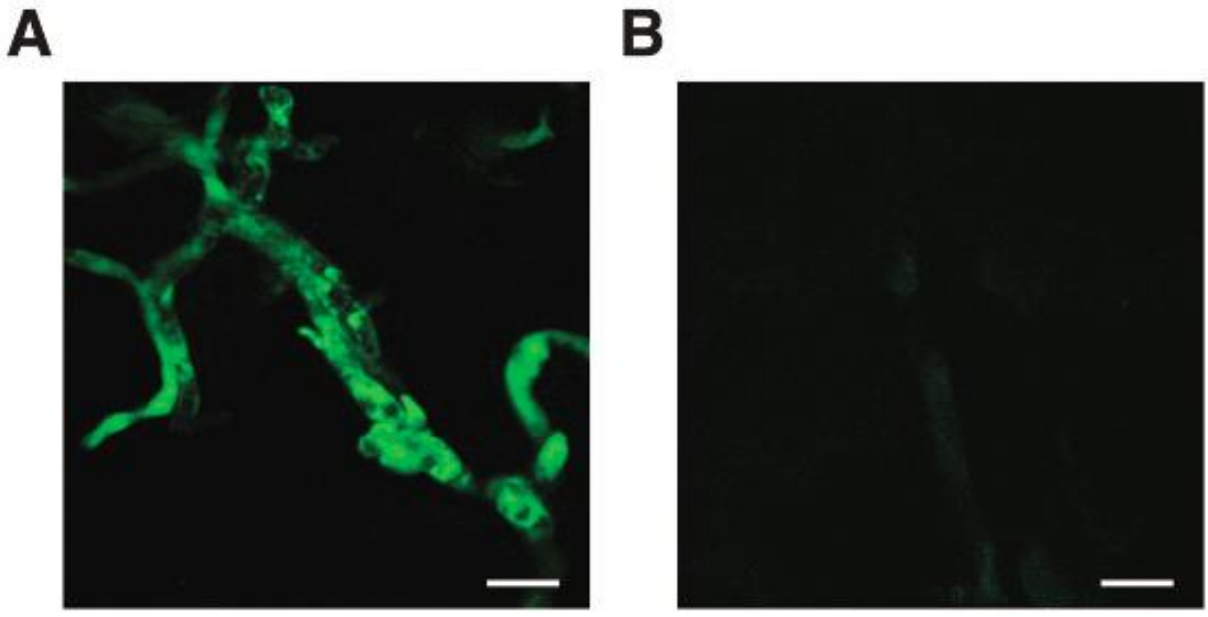
Early mycologists called the *Phytophthoras* 'the water fungi' due to their liking for and reliance on high humidity and surface water for spore germination, leaf infection, disease development, generation and liberation of spores and spread of late blight disease. As such the *P. infestans* pathogen is most active and late blight disease most severe in cool, wet conditions on outdoor tomato crops grown in temperate regions of the world and on tomato crops grown at higher elevations in the tropics.

MOLECULAR BIOLOGIST

Plant-mediated gene silencing restricts growth of the potato late blight pathogen *Phytophthora infestans*

Author: Jahan et al. (2015)
DOI: 10.1093/jxb/erv094

Abstract
Phytophthora infestans is an oomycete that causes severe damage to potato, and is well known for its ability to evolve rapidly in order to overcome resistant potato varieties. An RNA silencing strategy was evaluated here to clarify if small interfering RNA homologous to selected genes in *P. infestans* could be targeted from the plant host to reduce the magnitude of the infection. As a proof-of-concept, a hairpin RNA (hp-RNA) construct using the *GFP* marker gene was designed and introduced in potato. At 72 hpi, a 55-fold reduction of the signal intensity of a corresponding GFP expressing *P. infestans* strain on leaf samples of transgenic plants, compared with wild-type potato, was detected. This suggests that an RNA interference construct in the potato host could be processed and target a transcript of the pathogen. The *hp-PiGPB1* targeting the G protein β-subunit (*PiGPB1*) important for pathogenicity resulted in most restricted disease progress. Further, Illumina sequencing of inoculated transgenic potato leaves revealed sRNAs of 24/25 nt size homologous to the *PiGPB1* gene in the transgenic plants indicating post-transcriptional silencing of the target gene. The work demonstrates that a host-induced gene-silencing approach is functional against *P. infestans* but is highly dependent on target gene for a successful outcome. This finding broadens the arsenal of control strategies to this important plant disease.



Silencing of *GFP* in *P. infestans* by hp-RNA. Confocal laser scanning microscopy of *P. infestans* transformants (*Ham34:eGFP*) expressing green fluorescent protein. *GFP* expression in mycelia grown on (A) wild-type and (B) hp-GFPL1 (*UBQ:GFP-I-GFP*) transgenic potato. Bars=25 μm.

Terminology
hpi – hours post-inoculation
hp – hairpin

The cell biology of late blight disease
Author: Whisson et al. (2016)
DOI: 10.1016/j.mib.2016.09.002

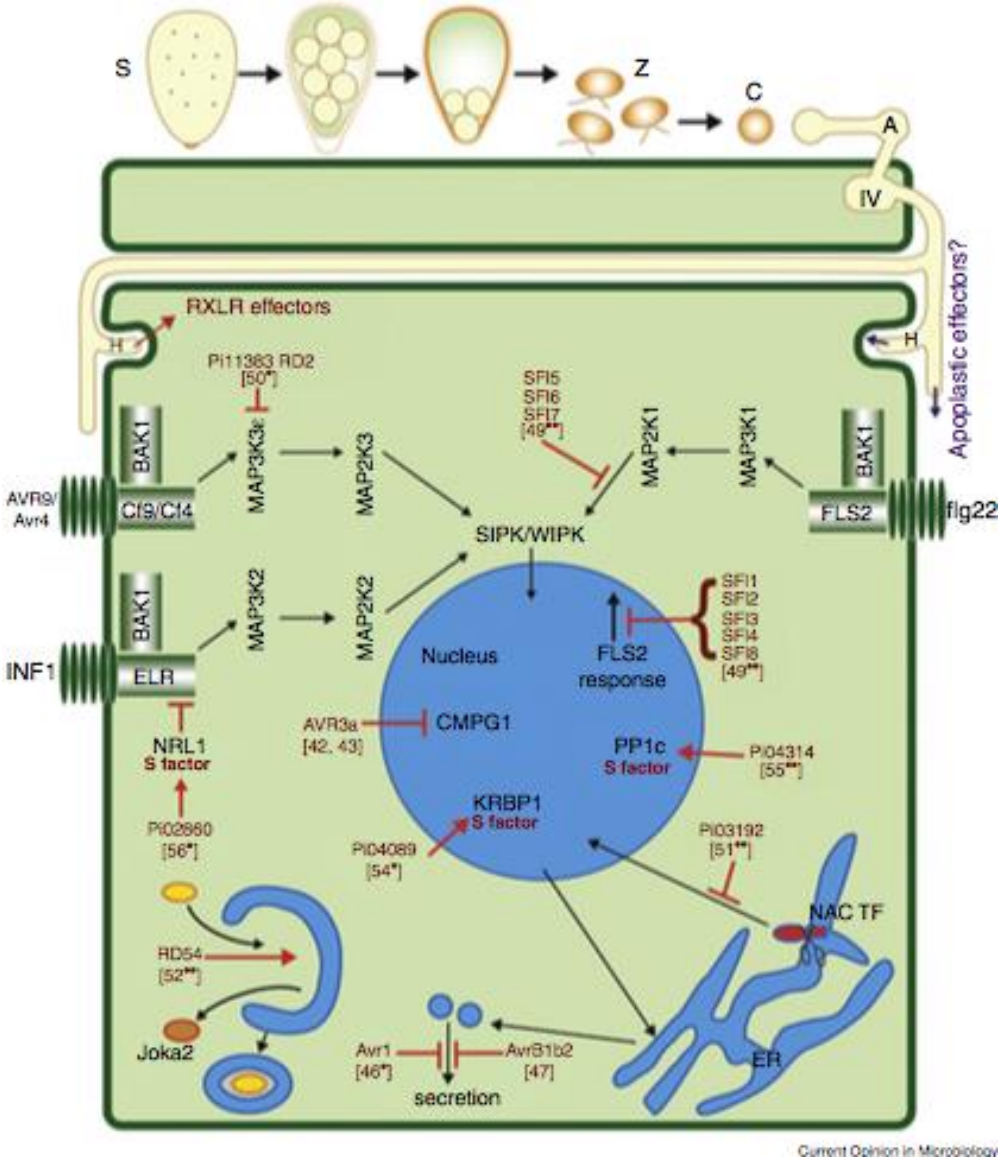


Figure 1. Diagram of the *P. infestans* infection cycle and the many roles effectors play in modulating plant cellular processes. The main dispersal stage is the multinucleate sporangium (S) which either germinates directly or releases zoospores (Z). Zoospores rapidly encyst (C) on a

host plant then germinate to form an appressorium-like (A) swelling at the end of the germ tube, under which penetration takes place to form the infection vesicle (IV). From this intercellular hyphae extend and grow between host cells, projecting haustoria (H) into cells. The haustoria are the sites of secretion of the RXLR class of effectors (shown in red) and some of their characterised protein targets and activities are represented in this diagram. Several effectors are attuned to suppress signal transduction pathways emanating from membrane-bound, BRASSINOSTEROID-ASSOCIATED KINASES 1 (BAK1)-dependent receptors such as FLAGELLIN-SENSING 2 (FLS2), and can act redundantly. Some effectors act to inhibit specific immune response factors and pathways, while others promote the activity of negative regulators which can thus be regarded as susceptibility factors (S factors). Several effectors target diverse nuclear-located processes while others target processes involving the endoplasmic reticulum (ER), vesicles in the secretory pathway, the plasma membrane, or autophagosomes.

Silencing of six susceptibility genes results in potato late blight resistance
Author: Sun et al. (2016)
DOI: 10.1007/s11248-016-9964-2

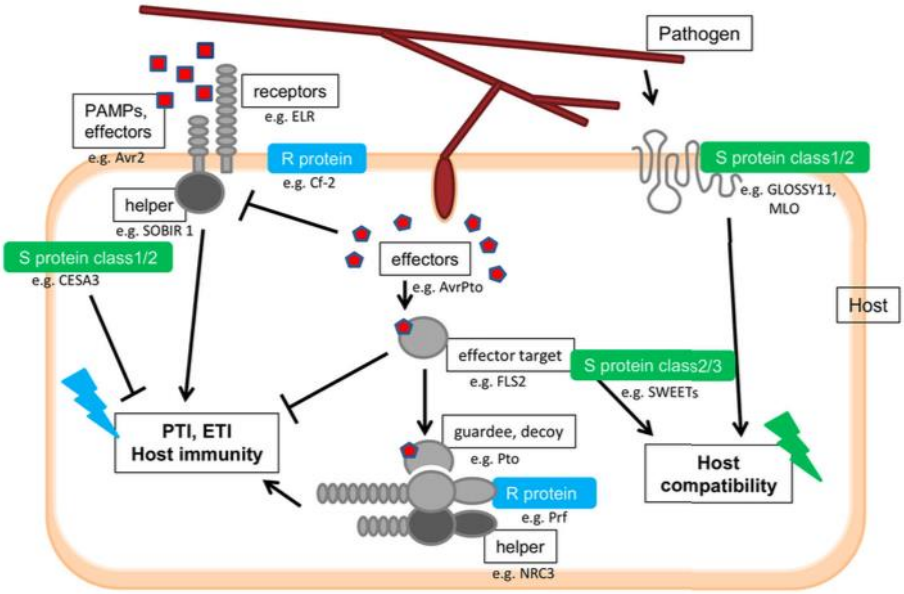


Fig. 1 Plant innate immunity: PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI). Apoplastic pathogen-associated molecular patterns (PAMPs/apoplastic effectors), intracellular effectors or modified effector targets are actively perceived by receptors in the plasma membrane or resistance (R) proteins in the cytoplasm, resulting in the activation of PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI). Helper proteins and guard proteins/ decoys are involved in the co-perception of pathogen-derived components (Césari et al. 2014). Pathogens use host proteins (S proteins) encoded by plant susceptibility genes (*S*-genes) to facilitate entry and growth, resulting in host compatibility (Doehlemann and Hemetsberger 2013). MLO, CESA3 and SWEETs are examples of S proteins in class 1, 2, or 3 according to van Schie and Takken (2014). Class 1 genes provide features that facilitate the entrance of a pathogen, class 2 genes increase innate immunity when the gene is disabled, and class 3 genes encode substrates essential for the pathogen

Introduction
The plant immune system comprises an intricate network of active and passive mechanisms that successfully prevent the colonization of a host by a pathogen (Jones and Dangl 2006) (Fig. 1). In many cases, defence is actively triggered upon first contact between a plant and pathogen.

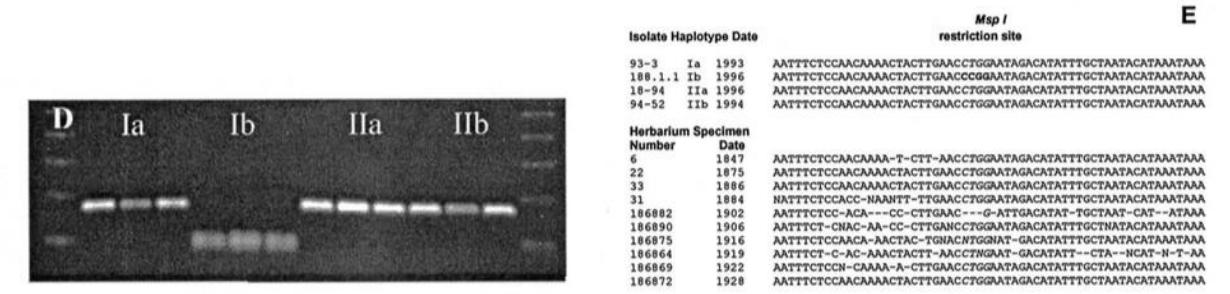
POTATO BLIGHT

Plasma membrane receptors perceive pathogen-associated molecular patterns (PAMPs) or apoplastic effectors (Fig. 1). This perception leads to intracellular signal transduction events, culminating in defence responses that, when effective, induce PAMP-triggered immunity (PTI). A known example is the receptor-like protein ELR (elicitin response). ELR was isolated from the wild potato species *Solanum microdontum* and can mediate the broad-spectrum recognition of elicitins (referred to as oomycete PAMPs) from several *Phytophthora* species (Du et al. 2015). The second layer of defence relies on proteins encoded by resistance genes (R-genes) that recognize intracellular avirulence (Avr) effectors. This recognition results in effector-triggered immunity (ETI).

Potato late blight, caused by *Phytophthora infestans*, is considered to be the most serious potato disease worldwide. An asexual cycle of sporangial proliferation is completed within 5 days (Fry 2008). Depending on environmental conditions, an unprotected potato field with a susceptible cultivar (cv.) can be devastated within 10 days after infection with *P. infestans* (Fry 2008). The control of potato late blight is dependent on fungicide sprays and the use of cultivars carrying dominant R-genes (Haverkort et al. 2009). All late blight R-genes identified thus far belong to the coiled-coil nucleotide binding leucine- rich repeat (CC-NB-LRR or NLR) class and reside inside the plant cell where these genes recognize *P. infestans* avirulence effectors (Avr) of the RxLR class.

Because the resistance conferred by R-genes is, in general, race-specific, ETI can be broken due to the rapid evolution of pathogen effectors in agricultural practice (Vleeshouwers et al. 2011). For example, Rpi- vnt1, isolated from *S. venturii*, confers resistance to a broad spectrum of *P. infestans* lineages and races in European potato growing areas (Pel et al. 2009). However, this resistance can be overcome by the EC1 lineage, which is abundant in Ecuador. Thus, in addition to exploiting dominant R-genes, combinations of resistance traits that are effective against the prevailing *P. infestans* population are needed for durable late blight resistance.

Tracking historic migrations of the Irish potato famine pathogen, *Phytophthora infestans*
Author: Ristaino (2002)



(D) *Msp*I-digested PCR products from amplification of mitochondrial DNA (mtDNA) from four haplotypes of *P. infestans* with primers pair P2 F4/R4. The *Msp*I site is found in Ib haplotypes but absent in the other haplotypes. (E) Mitochondrial DNA sequences around the *Msp*I restriction site in four modern and ten historic samples of potato infected with *P. infestans*.

EVOLUTIONARY BIOLOGIST

Plant-mediated gene silencing restricts growth of the potato late blight pathogen *Phytophthora infestans*

Author: Jahan et al. (2015)
DOI: 10.1093/jxb/erv094

Introduction
Phytophthora infestans is the oomycete pathogen responsible for the late blight disease on its potato host (*Solanum tuberosum*) inciting the worldwide most severe potato losses (Haverkort et al., 2008; Forbes, 2012). Enormous breeding efforts to produce new varieties with improved resistance have been ongoing for more than 100 years. Exploitation of resistance genes from wild *Solanum* species started with *S. demissum* (Reddick, 1928, 1934) and has continued ever since (Vleeshouwers et al., 2011).

A Survey of Tomato and Potato Fields in Florida Reveals Unique Genotypes of Phytophthora infestans between 2005 and 2007

Author: Schultz et al. (2010)

Introduction
Before the 1970s, populations *of P. infestans* in the United States and many countries of the world were predominated by the A1 mating type and some derivative of the US-1 genotype (Andrivon, 1996; Goodwin et al., 1994a). In Florida, late blight epidemics were infrequent until the early 1990s when conducive conditions likely allowed for the migration of new genotypes from Mexico (Goodwin and Drenth, 1997; Goodwin et al., 1995b). The first novel genotype observed in Florida was US-6 (A1 mating type) in 1991 (Goodwin et al., 1994a; Weingartner and Tombolato, 2004). Both US-6 and US-7 (A2 mating type) had been reported within a single field as early as 1993 (Goodwin et al., 1995b). In Florida, by 1994, the US-8 genotype had appeared and by 1996, the US-17 genotype and possible progeny of US-6 · US-8 was recovered from tomato (Goodwin et al., 1998). We suspect that novel genotypes may be responsible for severe late blight epidemics that have been occurring since 2005. The goal of the current study was to characterize the genotypes of *P. infestans* recovered from tomato and potato grown in production regions in southern and central Florida over multiple seasons.

Discussion
Our analysis of *P. infestans* isolates recovered from tomato and potato in Florida through growing seasons 2005 to 2007 identified unique genotypes not previously described within the United States. In 2005, isolates were similar to the US-13 genotype based on mating type, mtDNA, and isozyme profiles but could not be identified conclusively because a RG57 profile for US-13 genotype could not be obtained from the literature. Lacking a complete reference profile for US-13, we propose to recognize the 2005 isolates from tomato in Florida as US- 20 following the suggestions put forth previously (Forbes et al., 1998; Goodwin et al., 1994a). In 2006 to 2007, the US-20 clonal lineage was essentially displaced by a new genotype, and we propose the name US-21 for the clonal lineage collected in 2006 and 2007 in Florida.

POTATO BLIGHT

Late blight has been documented infecting potatoes in Florida as early as 1937 (Eddins, 1945). Likewise, *P. infestans* has been a persistent problem for tomato and potato growers Florida since 1993. This work was initiated as a result of an outbreak of late blight that occurred in the 2004–2005 (2005) growing season. This outbreak was unusual because the disease was particularly destructive to tomato, extremely difficult to control despite intensive spray programs, and it persisted through the end of the season. South Florida growers typically encounter late blight only during the coldest months of the season (December to March) and can generally achieve good control with fungicides. The growth chamber inoculations demonstrated that the US-20 isolates from 2005 caused statistically significant more disease severity on tomato under the same conditions when compared with US-21 isolates from 2006 (Fig. 2). All of the isolates produced symptoms on tomato, although the potato isolate Pi0639 caused the least disease severity in this test.

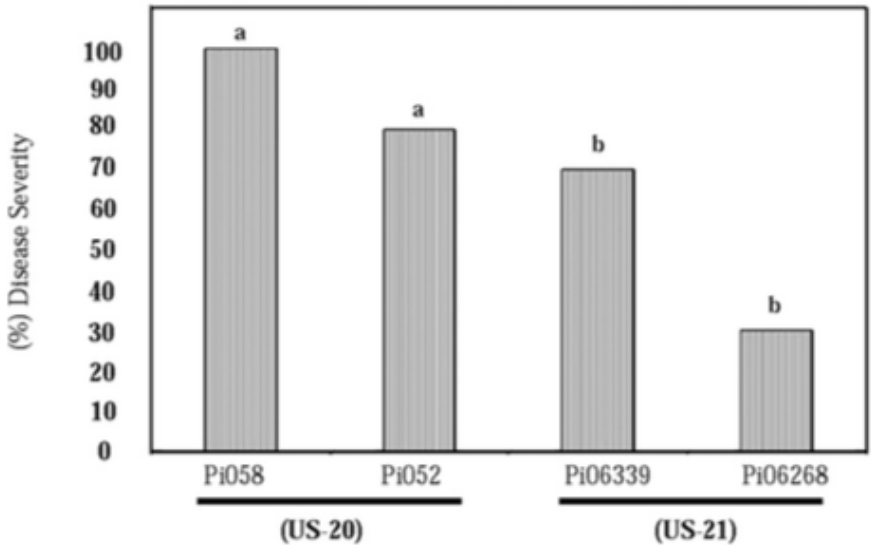


Fig. 2. Disease severity (% of infected tissue) of two US-20 (Pi058 and Pi052) isolates and two US-21 isolates (Pi0662 and Pi0628) inoculated on FL 47 tomato (mean of two experiments). Columns with different letters (a, b) are statistically different at $P < 0.0001$.

Late blight disease means early end for tomato crops
Author: Mabbett (2016)

Sexual reproduction in a versatile pathogen
Phytophthora infestans occurs as two mating types (A1 and A2) and if they come into contact, sexual reproduction may occur. A nucleus from the antheridium enters the oogonium and following karyogamy (the fusion of two nuclei) a thick-walled, diploid oospore is formed. Before the 1990s, only the mating type A1 was present in potato-growing areas outside of Mexico and therefore sexual reproduction did not play a significant role in the disease cycle. More recently, mating type A2 has migrated to most of the tomato and potato growing regions of the world and sexual reproduction is therefore thought to occur in an increasingly large number of more widely spread areas of the world.

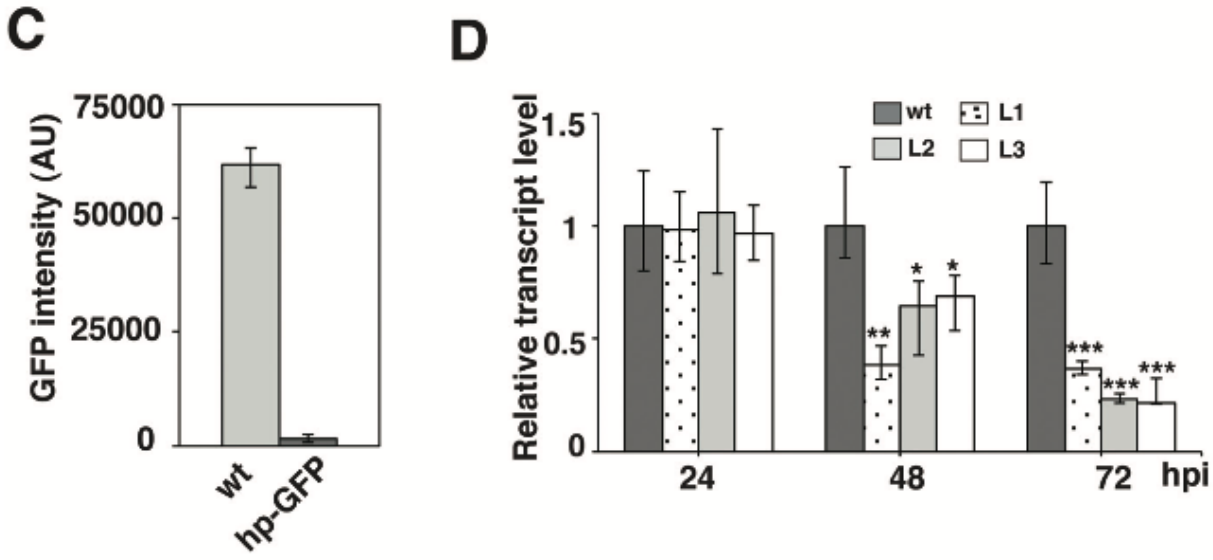
Epidemiology and integrated control of potato late blight in Europe

Author: Cook et al. (2011)
DOI: 10.1007/s11540-011-9187-0

Phytophthora infestans, the causal agent of late blight, is a major threat to potato production in northwestern Europe. Before 1980, the worldwide population of *P. infestans* outside Mexico appeared to be asexual and to consist of a single clonal lineage of A1 mating type characterized by a single genotype. It is widely believed that new strains migrated into Europe in 1976 and that this led to subsequent population changes including the introduction of the A2 mating type. The population characteristics of recently collected isolates in NW Europe show a diverse population including both mating types, sexual reproduction and oospores, although differences are observed between regions. Although it is difficult to find direct evidence that new strains are more aggressive, there are several indications from experiments and field epidemics that the aggressiveness of *P. infestans* has increased in the past 20 years.

STATISTICIAN

Plant-mediated gene silencing restricts growth of the potato late blight pathogen *Phytophthora infestans*
Author: Jahan et al. (2015)
DOI: 10.1093/jxb/erv094



Silencing of *GFP* in *P. infestans* by hp-RNA. (C) Fold change of total intensity of GFP in mycelia grown on hp-GFP plants compared with wild-type plants. AU=arbitrary unit. (D) Transcript abundance of *GFP* in *P. infestans* transformants grown on wild-type and transgenic plants at 24, 48, and 72 hpi, quantified by qRT-PCR. Data are normalized to *P. infestans actinA* mRNA levels and represent means ±SE (*n*=3 pooled leaves of 3 plants). Asterisks indicate significant difference to the wild type (Student's *t* test; **P* <0.05; ***P* <0.01; ****P* <0.001).

Terminology
hpi – hours post-inoculation
hp – hairpin

Late blight disease means early end for tomato crops
Author: Mabbett (2016)

Cultural and chemical control
Breeding of tomato varieties, which are resistant to late blight disease, is an increasing feature of the industry, especially in the south eastern United States where disease epiphytotics and complete loss of crops would be the norm, if were not for the use of a wide range of commercial fungicides.

Systemically acting, site-specific, action fungicides would have provided a major unfettered breakthrough in late blight control, by entering the plant and suppressing or curing established infections, if it were not for the ability of this genetically versatile microbial pathogen to

respond, through development of populations with resistance to specific fungicides and their chemistry. Having a 'site specific' action means systemic fungicides are better equipped to select out genetic variants within the pathogen population that possess a gene capable of directing the production of a specific enzyme capable of detoxifying or 'neutralizing' that fungicide's mode of action.

Capacity of the pathogen to develop resistance to a fungicide is made considerably more difficult when growers use contact protectant fungicides like cuprous oxide, which have multi-site action and correspondingly broad spectrum activity, right across the genetic profile of the pathogen population. In the 125 years of copper fungicide application and almost ninety years of using cuprous oxide there has been not one reported instance of resistance to the fungicidally active copper component in these compounds.

Today's tomato growers around the world now rely on an integrated approach to chemical control, by using site-specific, systemic fungicides, as stand-alone applications just once at the beginning of the season, in tank mixes of products with a completely different mode of action or applying alternating sprays of products with disparate chemistries. These strategies are aimed at reducing the risk of pathogen populations resistant to the action of specific fungicide chemistries developing in the field.

"What a Painfully Interesting Subject": Charles Darwin's Studies of Potato Late Blight
Author: Ristaino & Pfister (2016)
DOI: 10.1093/biosci/biw114

In the same letter, Darwin (1845a) said, "I think it is a very good suggestion of yours, about gentlefolk not buying potatoes & I will follow it for one. The poor people, wherever I have been, seem to be in great alarm: my labourer here has not above a few weeks consumption & those not sound; as he complains to me, it is a dreadful addition to the evil, flour being so dear: some time ago this same man told me, that when flour rose, his family consumed 15 pence more of his 12 s earnings per week on this one article. This would be nearly as bad, as if for one of us, we had to pay an additional 50 or 100 £ for our bread: how soon in that case, would those infamous corn laws be swept away."

Darwin's comments were set in a social and political context of rich and poor. The Irish famine disaster led to the repeal of the Corn Laws that had put high tariffs on imported grain. Darwin supported free trade. Darwin's concern about the rising cost of flour led him to an experiment with making potato flour, and when he purchased additional land, he "told [his] agent to arrange allotments to every laborer." The need for more allotments indicates the threat that late blight posed to food security of the rural poor who lacked their own land to grow crops. An expansion of the allotment system in England was under heated debate. In agreeing with Henslow, Darwin said that "gentle folks" should avoid eating potatoes so that the lower classes might have a larger supply of the carbohydrate-rich crop—natural selection at several levels, indeed.

Fitness costs associated with unnecessary virulence factors and life history traits: evolutionary insights from the potato late blight pathogen *Phytophthora infestans*
Author: Montarry et al. (2010)
DOI: 10.1186/1471-2148-10-283

Fitness (F) and aggressiveness components (LP, SD and LGR) were negatively correlated with virulence complexity (Figure 1) indicating additive fitness impacts of virulence factors.

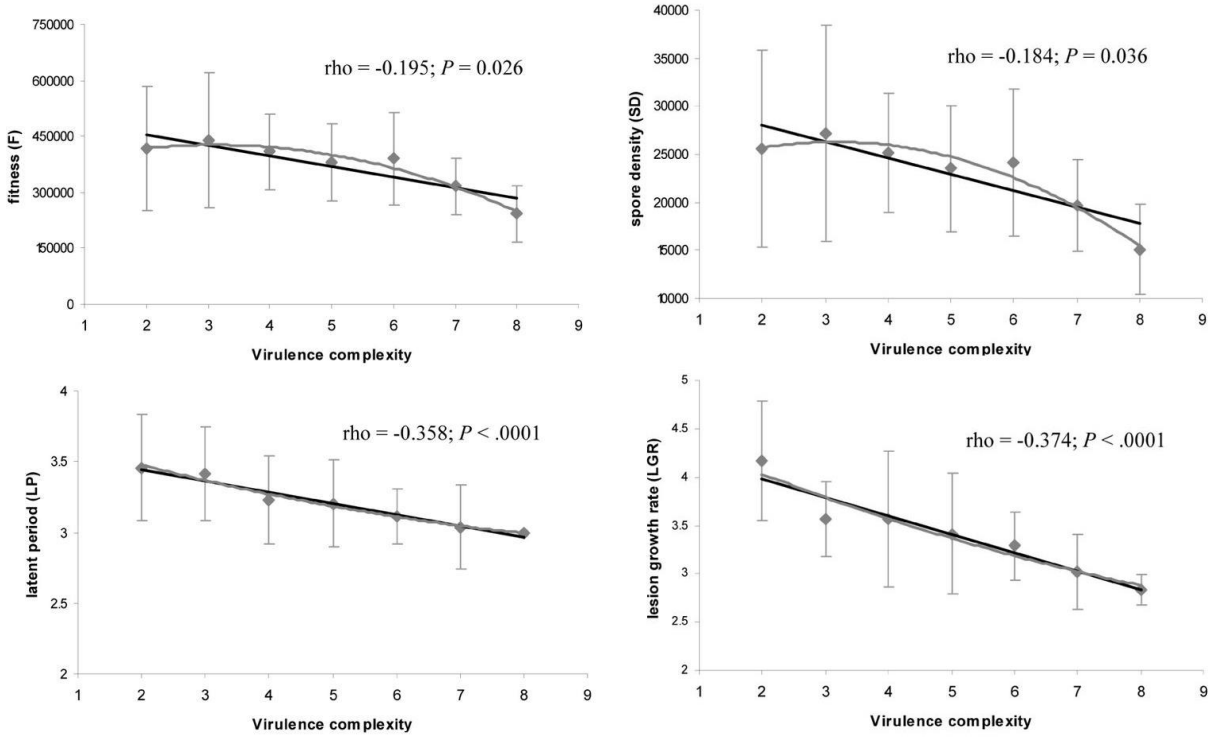


Figure 1
Relationship between fitness, latent period, spore production and lesion growth rate (mean values ± SEM) and virulence complexity (*i.e* . the number of R-genes overcome by the isolate) for the 132 *P. infestans* isolates tested on the susceptible potato cultivar Bintje.
Spearman's rank correlation rho and corresponding P-values were indicated on the graphs. Linear and quadratic regression models were indicated in black and grey, respectively.

Discussion
By contrast, our data, gathered on genetically related isolates, showed that fitness was negatively correlated with virulence complexity. This suggests that i) the small but consistent differences in aggressiveness observed for each individual gene are indeed cumulative, and may end-up in selection against the most complex races, and that ii) the rest of the pathogen genome (*i.e.* genes not directly related to pathogenicity) could be involved in the restoration of fitness costs due to unnecessary virulence.

IMMUNOLOGIST

The cell biology of late blight disease
Author: Whisson et al. (2016)
DOI: 10.1016/j.mib.2016.09.002

Introduction
Late blight is a devastating disease of potato and tomato. The organism that causes it, *Phytophthora infestans*, is an oomycete; related to diatoms and brown algae. Its discovery in the late 19th century contributed to the establishment of plant pathology as a research discipline. Late blight remains the number one potato disease nearly 150 years later. The global population of *P. infestans* constantly changes, with emergence of aggressive new strains, ensuring that late blight continues to be an ongoing threat to global food security [1,2].

There are over 120 known species of *Phytophthora* [3] and all are pathogens of plants. They colonise different host tissues, such as roots, tubers, herbaceous stems, woody trunks, foliage, and fruit. *Phytophthora* species develop distinct cellular stages in their infection cycle [4]. Multi-nucleate sporangia and uninucleate motile zoospores represent the primary dispersal stages. *P. infestans* sporangia formed on aerial plant parts may be blown or splashed to new hosts, where they may either germinate directly, or release zoospores, to initiate infection. Zoospores discard flagella and synthesise a cell wall, forming a cyst. These germinate within hours and may enter host tissue through natural openings such as stomata, or form an appressorium-like swollen germ tube, beneath which penetration of host epidermal cells occurs. Upon host cell penetration, a spherical primary infection vesicle is formed from which hyphae emerge to ramify through plant tissue. *P. infestans* hyphae grow intercellularly, projecting digit-like haustoria into host cells [5–8]. Haustoria are structures that form an intimate interaction with host cells, removing the plant cell wall but leaving the membrane intact to facilitate molecular exchange between the pathogen and a living plant cell. It is of interest to reveal how these specialised cell types differ from one another and how transition is regulated from one stage to the next: sporangium → zoospore → cyst → germination → appressorium → host penetration and infection vesicle → intercellular hyphal growth → haustorium formation → initiation of sporulation. Knowledge of the differences and similarities between these develop- mental stages will facilitate novel means to control infection through targeted inhibition of regulatory processes in the pathogen. Infection vesicles, haustoria and intercellular hyphae are of particular interest, as these stages are in close contact with plant cells and this is where the outcome of infection is determined (Figure 1).

Cell biology of protein localization in developmental stages
Microbes suppress plant immunity by the secretion of so-called effector proteins that can act either outside (apoplastic effectors) or inside (cytoplasmic effectors) the host cell. *P. infestans* is no exception.

Sites of action, targets and recognition of RXLR effectors inside host cells
PiAvr3a was the first RXLR effector from *P. infestans* to be studied in detail. Expressed transiently in *N. benthamiana* as an N-terminal FP fusion protein (CFP-PiAvr3a), lacking the secretion signal peptide, the effector is generally nucleo-cytoplasmic in host cells and retains its ability to suppress pathogen-associated molecular pattern (PAMP)- triggered immunity (PTI)

activated by perception of the *P. infestans* Infestin1 (INF1) protein. It does so by interaction in planta with the ubiquitin E3 ligase CMPG1, stabilising it and thus preventing its normal activity in promoting INF1-mediated cell death.

Plant-mediated gene silencing restricts growth of the potato late blight pathogen *Phytophthora infestans*
Author: Jahan et al. (2015)
DOI: 10.1093/jxb/erv094

Introduction
The success of *P. infestans* as a pathogen originates from its effective reproduction in both asexual and sexual forms. Under ideal conditions, the life cycle can be completed on foliage in about five days, where one lesion can generate up to hundreds of thousands of new sporangia (Fry, 2008) … Plants utilize post-transcriptional gene silencing to protect themselves against invasive nucleic acids such as transposons, viruses, and transgenes (Cogoni and Macino, 2000). Knowledge on the basic mechanisms of gene silencing provides new opportunities to explore plant–pathogen interactions and potential strategies for novel disease control. In this area there are promising reports where RNAi-based constructs in plants were designed to target fungal plant pathogens (Nowara *et al.*, 2010; Koch *et al.*, 2013; Ghag *et al.*, 2014). In order to expand the toolbox for potato breeders not just to rely on dominant resistance genes from various *Solanum* species, it would be interesting to know if a similar gene-silencing approach driven by the plant host could be a functional strategy to target this oomycete plant pathogen.

Results
To record overall plant performance, the plants were allowed to grow for a month. After 30 d, the inoculated wild-type plants were almost dead, whereas transgenic hp-PiGPB1 plants showed greatly reduced disease spread disease symptoms (Fig. 3C). A similar performance was seen for the hp-PiGAPDH transgenic plants (Fig. 3D), although the effect was not as prominent compared with the hp-PiGPB1 plants.

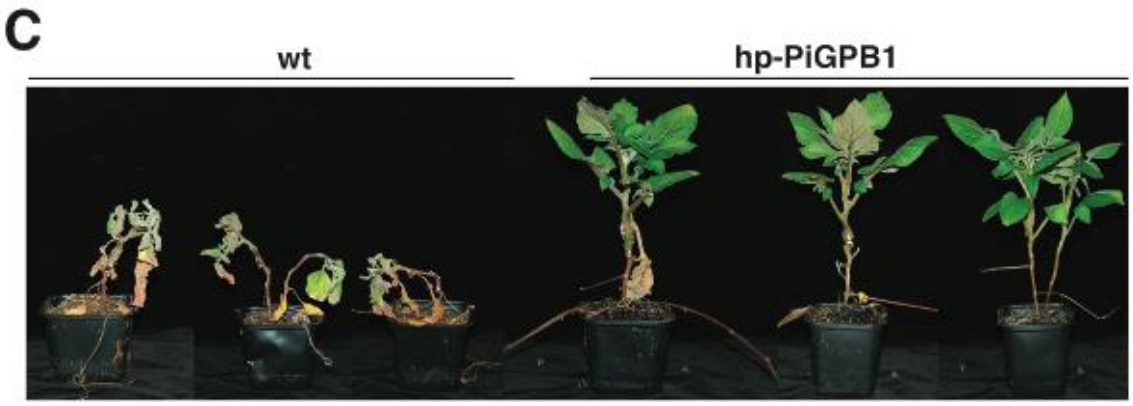


Fig. 3. Analysis of transgenic potato harbouring a *hp-PiGPB1* or *hp-PiGAPDH* construct. Overall performance of (C) wild-type and hp- PiGPB1 transgenic plants, and (D) wild-type and hp-PiGAPDH transgenic plants at 30 dpi.

Potential effects of diurnal temperature oscillations on potato late blight under climate change: Results from experiments and simulation modeling
Author: Shakya (2014)

Epidemiology of P. infestans
P. infestans is a hemibiotroph and thus requires living tissue for infection. When sporangia land on healthy leaf surface in the presence of enough moisture and optimum temperature conditions, infection takes place. Depending on temperature, the germination of sporangia is via a germ tube at temperatures more than 15°C and/or production of 6-12 motile zoospores below that temperature (Crosier 1934). The zoospore has two types of flagella to swim in free water, tinsel type and whiplash type. Effects of temperature on mode of germination have been studied intensively. Differences in germination have been reported for different clonal lineages … Crosier (1934) reported direct germination of sporangia at 18-20°C, … with the isolate of … US-1. Mizubuti and Fry (1998) reported … the newer clonal lineage US-8 germinates directly at 10-15°C, but not at 18-20°C. A Brasilian isolate (BR-1) is reported to have higher indirect germination percentage compare to US-1 when tested at 10°C and 15°C. Temperatures above 26°C are reported to have detrimental effects on sporangia germination Once the sporangia or zoospore has penetrated colonization of the host tissue takes place. Tiny lesions are visible within 2-3 days upon infection depending on temperature … Average temperatures ranging from 20-22°C favor symptom development (Andrade-Piedra et al. 2005b Crosier 1934; Hartill et al. 1990; Maziero et al. 2009; Mizubuti and Fry 1998).

Late blight disease means early end for tomato crops
Author: Mabbett (2016)

Disease epidemiology
Temperature and moisture are the most important environmental factors affecting the development of late blight disease on tomato crops. Sporangia are formed on the lower leaf surfaces and infected stems, even when relative humidity is less than 90%. Optimum range for spore formation (sporulation) is 18-22°C. Sporangia can germinate directly via a germ tube at 21-26°C but below 18°C zoospore production kicks with each sporangium producing 6 to 8 zoospores and requiring water for swimming on the plant surface.

Each zoospore can initiate an infection and this explains why the disease moves more quickly and is much more severe during cool, wet conditions. Cool nights, warm days, and extended wet conditions caused by rain, mist and fog, and regularly encountered in the tropical highlands, will invariably result in late blight epiphytotics (epidemics) during which entire fields of tomato, not protected by fungicides, can be destroyed in a matter of days.

HISTORICAL CONTEXT

History of biological warfare and bioterrorism

Author: Barras et al. (2014)
DOI: 10.1111/1469-0691.12706

Among the main concerns during the contemporary period is undoubtedly the possibility of the use of biological weapons in the context of bioterrorism in a strict sense, i.e. the use of biological weapons by non-state-sponsored individuals or groups. From the 1980s on, one striking example is offered by the Rajneesh cult, a religious group who, in 1984, intentionally contaminated salad bars with *Salmonella typhimurium* in various restaurants in Dalles, Oregon. This attack, which resulted in 751 cases, 45 of whom had to be hospitalized, seems to be one of the very few confirmed instances of biological terrorism after World War II, with a few exceptions such as the 'anthrax letters case'.

Discernment between deliberate and natural infectious disease outbreak

Author: Dembek et al. (2007)
DOI: 10.1017/S0950268806007011

Salmonellosis is the second most common food-borne illness [6], and contaminated food (most often poultry) is the principal route of disease transmission [7]. Salmonellosis manifests as acute gastroenteritis with fever. Occasionally more severe manifestations occur, especially in the very young or elderly.

In 1984, two large cohorts of Salmonella cases occurred in The Dalles, Oregon. The size and nature of this outbreak initiated a criminal investigation. The cause only became known when the Federal Bureau of Investigation (FBI) investigated a nearby cult (Rajneeshees) for other criminal violations [8]. In October 1985, a vial containing a culture of *Salmonella typhimurium* was discovered by authorities in the Rajneeshee clinic laboratory [9]. This strain was indistinguishable from the outbreak strain as isolated from food items and clinical specimens; records were found documenting its purchase prior to the outbreak.

Averting Apocalypse at Rajneeshpuram

Author: Goldman (2009)
DOI: 10.1093/socrel/srp036
Rajneeshpuram began in the early 1980s, when Bjagwan Shree Rajneesh and about 2,000 of his sannyasins (devotees) created the communal city of Rajneeshpuram on the Big Muddy Ranch about 150 miles east of Portland in sparsely populated Wasco County, Oregon. The devotees who settled in Oregon were primarily from the United States, although there were small contingents of western Europeans and Australians. They hoped to blend spirituality and materialism, while building an intentional community that could also serve as a destination resort and pilgrimage center for sannyasins from all over the world, supplanting the group's previous ashram in Poona, India.

SALMONELLA

Rajneesh kept a vow of public silence for three years, but he appeared for a daily afternoon drive in one of his 96 Rolls Royces, waving to sannyasins that lined up along the road. With the exception of the drives, the guru retreated from public view, delegating organizational leadership to Ma Anana Sheela, his personal secretary.

From the moment sannyasins settled in Oregon, they challenged established laws and customs, generating a range of opposition throughout the state. The most controversial incidents occurred in autumn of 1984 when Sheela and her inner circle bussed in hundreds of homeless individuals, mostly men, in a futile effort to control county elections. Massive negative publicity, state monitoring of voter registration, and legal opposition doomed the plan. By the end of 1984, almost all of the estimated 1,500 homeless visitors departed.

Less than a year after the "Share a Home" debacle, Sheela and her inner circle fled Rajneeshpuram for Europe. As his community disintegrated, Rajneesh spoke publicly once again, accusing Sheela and her circle of drugging dissident sannyasins, wire-tapping, arson, attempted murder, and embezzlement of Rajneesh movement funds. Most important, Rajneesh publicly revealed that Sheela ordered a few members of her inner circle to sprinkle salmonella bacteria in almost a dozen restaurant salad bars located in Wasco County, poisoning at least 750 individuals. It was a test run for a more massive effort that could temporarily incapacitate large numbers of anti-Rajneesh voters on election day.

Biopreparedness in the Age of Genetically Engineered Pathogens and Open Access Science: An Urgent Need for a Paradigm Shift
Author: MacIntyre (2015)

In September 1984, a large epidemic of salmonella occurred in the United States, with 751 cases arising from 10 restaurants. Eating at the salad bar was identified as the risk factor. Health authorities concluded this was a food-borne outbreak, and the cause, unsanitary food handlers. The salad bars were closed down, the outbreak subsided, and the matter would have ended there, but for a local politician, Jim Weaver, who accused a local religious cult of deliberately contaminating salad bars.

He went to the media with his claims, but health authorities refuted him, and he was branded paranoid and prejudiced against the religious cult. Six months later, the leader of the cult, Bhagwan Shree Rajneesh, confessed to the attack. Initially, his confession was not believed, but a year after the outbreak the Federal Bureau of Investigation (FBI) found an exact genetic match of salmonella in the cult's laboratories.

The outbreak occurred in Oregon in 1984. Rajneesh had purchased a ranch for 4,000 followers, and was in conflict with Wasco County over land use, and sought to take control of the County by making enough locals sick so that they would not be able to vote. The restaurant attack was a practice run, with the final plan to contaminate the town water supply before election day. Numerous cult members were involved, including 2 registered nurses. This case was not discussed publicly or written up in medical literature for another 12 years.

SALMONELLA

There are only two explanations for 10 restaurants being simultaneously affected by an identical strain of salmonella— either a common contaminated ingredient in all affected restaurants, or deliberate contamination. Yet neither explanation was considered, despite Jim Weaver's warning. Even when Rajneesh confessed, he was not believed. This case study illustrates the inability of experts to interpret the data correctly, and the active resistance to considering bioterrorism as a cause. The normal human tendency is to force available facts to fit the dominant paradigm of thinking. Most food borne outbreaks are because of unsafe food handling, and this is the dominant paradigm for a field epidemiologist. Even experts may default to the dominant paradigm and override fact with belief. There is a need to train field epidemiologists to recognize unusual patterns and consider bioterrorism as a possibility. If the possibility is never entertained, it will never be detected. The Rajneesh case would have remained undetected if not for the unsolicited confession. The ridiculing of the 1 person who recognized bioterrorism and the silence around the case for more than a decade illustrate important lessons.

MOLECULAR BIOLOGIST

A Large Community Outbreak of Salmonellosis Caused by Intentional Contamination of Restaurant Salad Bars

Author: Török et al. (1997)

Salmonella typhimurium was isolated from stool specimens of 388 patients (52%). The outbreak strain did not ferment dulcitol, which is an unusual biochemical characteristic found in only about 2% of nontyphoidal salmonellae. The outbreak strain was sensitive to ampicillin, cephalothin sodium, chloramphenicol, gentamicin sulfate, kanamycin sulfate, nalidixic acid, sulfisoxazole, and trimethoprim-sulfamethoxazole. Intermediate sensitivity was noted to tetra cycline and streptomycin sulfate. Plasmid profiles were determined for 52 outbreak-associated isolates, including an isolate from at least 1 case employee and 1 case customer from each group 1 restaurant. All outbreak isolates had the same plasmid profile, with a single plasmid of approximately 60 Md.

Salmonella Typhimurium was isolated from blue cheese salad dressing collected from restaurant B during the second wave of the outbreak, but was not isolated from dry mix used to prepare the dressing. *Salmonella typhimurium* was not isolated from cultures of lettuce from restaurants D and G, which came from the same lettuce shipments used during the outbreak. None of 6 *S. typhimurium* isolates collected in Oregon from sporadically occurring cases between July and December 1984 resembled the outbreak strain from The Dalles.

The RNA chaperone Hfq is essential for the virulence of *Salmonella* Typhimurium

Author: Sittka et al. (1997)
DOI: 10.1111/j.1365-2958.2006.05489.x

The bacterial Sm-like protein, Hfq, has been increasingly recognized as a post-transcriptional regulator of global gene expression. … its importance in uninfected bacteria remained unclear until it was shown that an *hfq* insertion mutant of *E. coli* exhibited broad, pleiotropic phenotypes affecting growth rate, cell morphology and tolerance of stress conditions. Several recent studies addressed a potential role of Hfq in the virulence of pathogenic bacteria. A *Brucella abortus hfq* mutant displayed significantly reduced survival in cultured murine macrophages, and attenuated virulence in a mouse model (Robertson and Roop, 1999). Similarly, Hfq was reported to be essential for the virulence of *Vibrio cholerae* … the pathogenesis of *Listeria monocytogenes* in mice (Christiansen *et al*., 2004), *Legionella pneumophila* virulence in amoeba and macrophage infection models, … [and] *Pseudomonas aeruginosa* [as an opportunistic pathogen in humans].

Based on the sequence data, three *hfq* mutant or control strains were constructed in SL1344 to study Hfq functions *in vivo* (**Fig. 1B**). In the D*hfq* mutant, the entire *hfq* coding region is replaced by a *cat* (chloramphenicol resistance, Cm^R) marker. As the *cat* gene used here does not carry a transcriptional terminator, transcription of the poly-cistron should be unaffected. *hfq*-C is a control strain in which the *cat* gene is inserted after the *hfq* stop codon. In control strain *hfq*HIS, the *cat* gene is inserted before the UAA stop codon. In addition, this latter insertion adds

six histidine codons to the last *hfq* codon, thus producing a chromosomally encoded His-tagged Hfq protein.

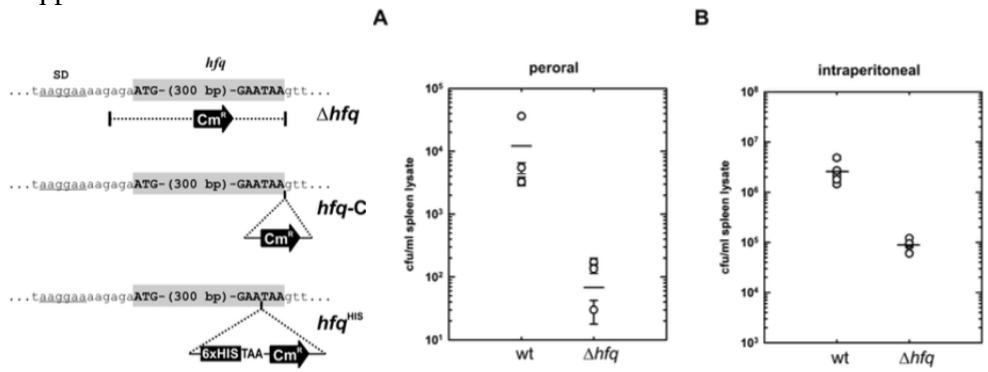


Figure 1B. (Left) Schematic representation of the insertion sites of the *cat* resistance cassette in the deletion mutant D*hfq*, the control strain *hfq*-C, and the chromosomally HIS-tagged strain, *hfq*HIS. **Figure 2. (Right)** The D*hfq* mutant is severely attenuated in mice. **A.** Groups of five Balb/c mice were infected perorally (by mouth) with suspensions of ~108 bacteria of either the wild-type or D*hfq* strains. Bacterial loads in spleen homogenates were determined 72 h post infection. For intraperitoneal infections (**B**) 1:1 mixtures of both, wild-type and D*hfq* strain, each strain at ~105 bacteria, were used for infections. Forty-eight hours post infection, spleens were removed and the cfu ml-1 for each strain was determined in spleen homogenates

Mice were also co-infected intraperitoneally with a mixture of the wild-type and D*hfq* strains, where uptake by resident macrophages should circumvent the need for invasion. Two, independent experiments indicated that the *hfq* mutant showed at least a 30- to 100-fold reduced uptake and/or survival in macrophages and subsequent carriage to the spleen compared with the wild-type strain (**Fig. 2B**), leading to calculated competitive indices (CI; Shea *et al*., 1999) of 0.01–0.03. This is consistent with the idea that both uptake and intracellular survival/proliferation in macrophages were affected.

Evolution of *Salmonella enterica* Virulence via Point Mutations in the Fimbrial Adhesin
Author: Kisiela et al. (2012)
DOI: 10.1371/journal.ppat.1002733

SNPs that represent random spontaneous mutations in the coding or regulatory regions of genes can result in modification or loss of gene function and/or expression [27]. These so called 'change of function/loss of function' mechanisms can confer a strong selective advantage to bacteria during their spread and growth in diverse host environments, improving their survival or increasing their pathogenic potential and thus driving their evolution toward an enhanced pathogenic phenotype. Therefore they are referred to as pathoadaptive mutations [27]. A well characterized example of pathoadaptative mutation is allelic variation in the FimH adhesin of type 1 fimbriae expressed by uropathogenic *Escherichia coli* (UPEC)[28]. FimH mediates mannose-sensitive bacterial adhesion to target cells [29]. It has been demonstrated that uropathogenic isolates, due to structural point mutations in *fimH*, express variants of FimH with an increased capability to bind monomannose receptors, conferring a significant advantage for colonization of the bladder compared to most commensal *E. coli* [30,31]. This adhesive protein has been reported to play an important role in Salmonella adhesion and invasion [36,37,38,39,40,41,42], and

recently was shown to be a crucial mediator of bacterial transcytosis through M-cells, or [microfold cells in the gastrointestinal tract], a process which is of great relevance in triggering the mucosal immune response.

***Salmonella enterica* serovars Typhimurium and Typhi as model organisms revealing paradigm of host-pathogen interactions**
Author: Garai et al. (2012)
DOI: 10.4161/viru.21087

Type three secretion systems. Type III secretion systems (T3SS) are present on the cell wall and possess a needle like structure. … The T3SS are dedicated to secrete certain proteins which bring about specific effects in the microenvironment of the cell.

Salmonella pathogenicity island 1 (SPI-1). SPI-1 plays pivotal role in both forms of diseases caused by *Salmonella*, i.e., gastroenteritis as well as systemic infection.[23] It carries out multiple functions, which include cytotoxicity of macrophages,[24] invasion of epithelial cells,[25] inflammation and fluid secretion in ileum[26] and cytokine response. SPI-1 also induces apoptosis in macrophages[24].

Adhesins. The mere attachment of the bacterium to the target cell consists of many steps mediated by various adhesins encoded either by fimbrial genes like type 1 fimbriae (*fim*),[41] plasmid encoded fimbriae (*pef*),[42] … [among others.] Each adhesin belonging to this pool is assigned to mediate adhesion to particular kind of cells due to specificity for the receptors present on the surface of these cells,[48] for example, … type 1 fimbriae *fimH* mediates attachment to dendritic cells.[49]

Plasmid encoded virulence genes. Salmonella possesses extra-chromosomal genes which are equally important for infection. For example, *Spv* works in a SPI-2 dependent manner and is essential for virulence of *S.* Typhimurium[51].

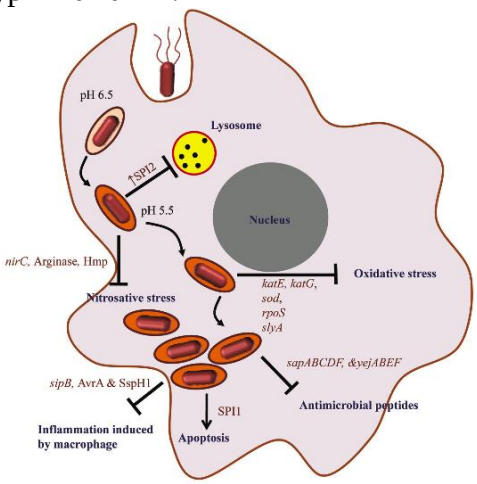


Figure 3. Immune evasion strategies of *Salmonella*. The intracellular life-cycle of *Salmonella* includes the entry of the bacterium in the host cell, SCV formation (whose pH changes from 6.5 to 5.5 depicted by change in the color of SCV compartment), evasion of host immune response and ultimately host cell death by apoptosis. The text in dark blue shows the immune responses

and processes within the host cell that take place during *Salmonella* infection and text in dark red depicts the factors that help *Salmonella* to evade these immune responses.

EVOLUTIONARY BIOLOGIST

Evolution of *Salmonella enterica* Virulence via Point Mutations in the Fimbrial Adhesin
Author: Kisiela et al. (2012)
DOI: 10.1371/journal.ppat.1002733

Salmonella enterica is comprised of six subspecies (I, II, IIIa, IIIb, IV and VI) further subdivided into, 2,500 serovars based on the presence of distinct surface antigens (somatic O, flagellar H and capsular Vi). The vast majority of *Salmonella* strains pathogenic to humans belong to subspecies I (*S. enterica* subsp. enterica), which is considered to be adapted to warm-blooded animals unlike the remaining subspecies which are found mostly in reptiles [1,2]. However, among, 1,500 serovars of subspecies I, relatively few cause severe systemically invasive infections while most serovars cause milder infections, usually limited to gastroenteritis. Heterogeneity in *Salmonella* virulence has been traditionally attributed to different distributions of various mobile genetic elements such as chromosomal pathogenicity islands, bacteriophages, transposons, plasmids, etc. … In this study, however, we demonstrate that amino acid mutations in core genes of Salmonella are also a driving force behind pathoadaptive evolution of *Salmonella* serovars.

The evolution of pathogenic *Salmonella* from a non-pathogenic ancestor is primarily attributed to virulence genes acquired by horizontal gene transfer [3]. This includes the acquisition of large chromosomal regions (10–200 kbp) called *Salmonella* pathogenicity islands (SPI) that contain a number of functionally related genes [19]. Acquisition of small (<5 kbp) genetic loci, bacteriophages, and plasmids also contribute to the evolution of virulence [4,5]. At least five *Salmonella* pathogenicity islands (SPI-1 to -5) have been identified in the serovars of *S. enterica* species, with a further nine islands with characteristics of SPIs identified in genomes of different serovars of subspecies I [20,21].

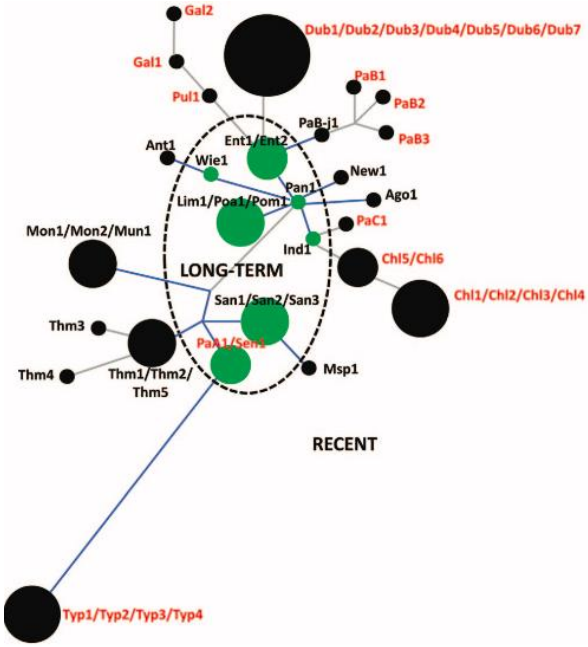


Figure 3. DNA-based protein phylogram of *S. enterica* FimH, derived from ZP analysis. The tree was built based on the 50 *fimH* sequences of *S. enterica* subsp. I. Each circle represents a

unique structural variant, and the size of the circle is proportional to the number of representative sequences. The dashed line separates the long-term (green) from the recently emerged variants (black). Branches marked in blue indicate branches containing synonymous mutations. The length of each branch is proportional to the number of non-synonymous mutations that were acquired. The strain tags of systemically invasive serovars are in red and the non-invasive serovars in black.

Mucosal inflammatory response to *Salmonella typhimurium* infection
Author: Patel & McCormick (2014)
DOI: 10.3389/fimmu.2014.00311

The architecture of the mucosal immune system, including mucins, antimicrobial peptides, resident microbiota, paracellular junctions, and effector cells of the lamina propia, functions to prevent pathogenic bacteria from disrupting the epithelial cell monolayer and causing disease. If enteric pathogens are able to penetrate these barriers, then it results in a host inflammatory response and eventually activation of an adaptive immune response, designed to eradicate the intruding pathogen. However, *S. typhimurium* has evolved systems, namely the SPI-1 and SPI-2 T3SS, to manipulate the defensive mechanisms of the mucosal immune system in order to develop a replication niche in the mucosal epithelium. Additionally, the ability of *S. typhimurium* to exploit inflammation allows it to penetrate the epithelial barriers, a condition in which activation of the adaptive immune response would be required for pathogenic clearance.

Molecular evolution of *Salmonella enterica* serovar Typhimurium and pathogenic *Escherichia coli*: From pathogenesis to therapeutics
Author: Lavigne & Blanc-Potard (2008)
DOI: 10.1016/j.meegid.2007.11.005

Commensal bacteria, as *Escherichia coli*, have adapted to coexist with the human host without causing disease. Pathogenic bacteria have adapted to colonize the human host and have acquired the ability to cause clinically significant pathologies. *Salmonella enterica* and *E. coli* are closely related enterobacteria that diverged from a common ancestor 100 – 150 million years ago (Doolittle et al., 1996). The genomes of the two species are essentially superimposable and genome sequencing demonstrated that the median homology between non-pathogenic *E. coli* and *Salmonella enterica* serovar Typhimurium (*S. typhimurium*) genomes is 80% Both species have evolved into intestinal pathogens. In addition, *E. coli* strains have also evolved as extraintestinal pathogens. The molecular evolution of virulence of human pathogens *S. typhimurium* and pathogenic *E. coli* is driven by the acquisition of multiple genetic elements (pathogenicity islands, plasmids, and pro-phages) by horizontal gene transfer. These horizontally acquired elements encode virulence factors necessary for colonization and replication within the host, neutralization of host defences and spread into new hosts. Main virulence determinants used by *S. typhimurium* and/or *E. coli* include adhesins, type III secretion systems (T3SS) that inject effector proteins into host cells, toxins and iron acquisition systems. … Genes that are stable in pathogenic strains, and absent from non-pathogenic strains, can provide novel therapeutic targets and novel vaccine strategies.

SALMONELLA

Non-typhoidal salmonellosis is one of the most common food-borne bacterial diseases in humans in industrialised countries. Humans are infected through contaminated food and water. The most commonly *S. enterica* serotypes in humans are *S. typhimurium* and *S. enteritidis*, which account for more than 75% of reported cases.

S. typhimurium and pathogenic *E. coli* genomes are characterized by the presence of virulence determinants that have been acquired by lateral [horizontal] gene transfer. In both species, these genetics elements, which are mobile or formerly mobile, include pathogenicity islands, prophages, and plasmids. [Pathogenicity islands] is commonly used to describe chromosomal regions that contain virulence genes and that are absent from non-pathogenic strains of the same or closely related species. Although PAIs differ in structure and function, they share several common features including the fact that they are frequently inserted at tRNA genes, that their G + C content differs from the rest of the genome and that they encode remnants of mobile elements. Mobilizable plasmids and bacteriophages represent key elements that enable bacteria to exchange genetic information through conjugation and transduction. Prophages are integrated in the bacterial genome in a process known as phage lysogenic conversion.

Evolution and Ecology of Salmonella

Author: Winfield & Groisman (2007)
DOI: 10.1128/ecosalplus.6.4.6

The most apparent difference between nonpathogenic commensal *E. coli* and pathogenic *Salmonella* is the presence of species-specific genes [13]. There are >1,100 genes in the *S. enterica* serovar Typhimurium LT2 genome that are absent from the *E. coli* K-12 genome, and >800 *E. coli* K-12 genes lack homologues in the serovar Typhimurium LT2 genome [6]. Serovar Typhimurium LT2 and *E. coli* K-12 share 55% of their proteins, which is higher than the 39.2% of proteins shared among *E. coli* K-12, enterohemorrhagic *E. coli* EDL933, and uropathogenic *E. coli* CFT073 [10]. A majority of *Salmonella* subspecies harbor 10 distinct regions, called *Salmonella* pathogenicity islands (SPI), not found in *E. coli*, along with numerous smaller genomic islets [7, 14, 15, 16]. The ancestral *Salmonella* lineage evolved following the acquisition of several genetic elements required for host cell invasion and induction of diarrhea in the infected animal

The Kauffmann-White serotyping scheme, which is based on the antigenic variation in surface molecules, has been used to identify and classify *Salmonella* strains for over 60 years [30]. The extensive antigenic variation in the somatic lipopolysaccharide (O) antigen, the phase 1 and phase 2 flagellar (H1 and H2, respectively) antigens, and the capsular (Vi) antigen (which is specific to serovar Typhi) can be used to distinguish >2,300 serovars [24, 27].

Flagellin gene sequence evolution in *Salmonella*
Author: Mortimer et al. (2007)
DOI: 10.1016/j.meegid.2006.12.001

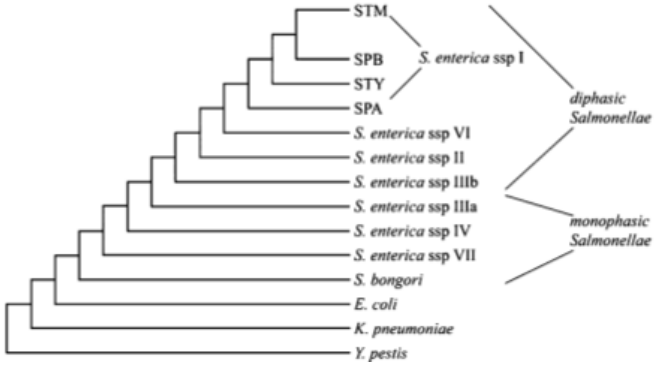


Figure 4. Phylogenetic tree of the *Salmonella* clade. The depicted cladogram was generated based on genes acquired and retained in *Salmonella* evolution. *S. bongori*, the oldest of the Salmonellae, acquired a second pathogenicity island to give rise to *S. enterica*, which diverged into subspecies.

STATISTICIAN

A Large Community Outbreak of Salmonellosis Caused by Intentional Contamination of Restaurant Salad Bars
Author: Török et al. (1997)

We identified 751 patients who met the case definition; 441 patients (59%) were female and 310 (41%) were male. Patients ranged in age from newborn to 87 years (median, 33 years). At least 45 persons (6%) were hospitalized; no fatalities were reported. The epidemic curve was biphasic (Figure 1). The first wave of illness, September 9 through 18, peaked on September 15, and the second wave, September 19 through October 10, peaked on September 24. Of 674 patients (90%) with known date of symptom onset, 88 (13%) became ill during the first wave and 586 (87%) became ill during the second wave. There were 692 restaurant-associated cases (92%), 11 secondary cases (1%), and 48 cases (6%) with incomplete information on restaurant exposure (Table). Among persons with restaurant-associated cases, 101 (15%) were employees and 591 (85%) were customers. There were 519 single-restaurant exposure (SRE) case customers and 72 case customers with multiple restaurant exposures.

Ten of the 38 restaurants in The Dalles were definitely affected (group 1) (**Table**). Two group 1 restaurants had culture-confirmed SRE case customers in the early wave (restaurants A and B), but all 10 were affected in the late wave. These 10 restaurants were associated with 494 SRE case customers (95%), 69 case customers with multiple restaurant exposures (96%), and 91 case employees (90%). Twelve restaurants were possibly affected (group 2), accounting for 25 SRE case customers (5%). Three case customers with multiple restaurant exposures (4%) had reported eating at a group 2 restaurant but not at a group 1 restaurant. Sixteen restaurants were not affected (group 3). There was no geographic clustering of affected restaurants, but dates of exposure for culture-confirmed cases were clustered. Restaurant involvement in the outbreak was associated with operating a salad bar.

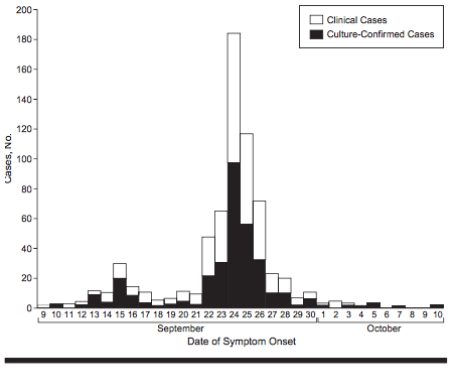


Figure 1.—Reported cases of *Salmonella* Typhimurium gastroenteritis by date of symptom onset for 674 cases (89.8%) with known date of onset, The Dalles, Ore, 1984.

Outbreak-Associated Cases of *Salmonella* Gastroenteritis by Group and Exposure Location

Group and Exposure Location	Culture-Confirmed Cases, No. (% of Total)	Clinical Cases, No. (% of Total)	Total Cases, No. (% of Total)
Case employees			
Group 1 restaurants (n=10)	74 (9.9)	17 (2.3)	91 (12.1)
Group 2 restaurants (n=12)	4 (0.5)	5 (0.7)	9 (1.2)
Group 3 restaurants (n=16)	1 (0.1)	0 (0.0)	1 (0.1)
Case customers			
Group 1 restaurants (n=10)	227 (30.2)	267 (35.6)	494 (65.8)
Group 2 restaurants (n=12)	6 (0.8)	19 (2.5)	25 (3.3)
Group 3 restaurants (n=16)	0 (0.0)	0 (0.0)	0 (0.0)
Multiple restaurant exposures*	32 (4.3)	40 (5.3)	72 (9.6)
Secondary cases	4 (0.5)	7 (0.9)	11 (1.5)
Cases with incomplete information	40 (5.3)	8 (1.1)	48 (6.4)
Total	**388 (51.7)**	**363 (48.3)**	**751 (100.0)**

*A total of 69 (95.8%) of 72 case customers with multiple restaurant exposures reported eating in 1 or more group 1 restaurants.

During the criminal investigation, testimony by commune members indicated that the outbreak in The Dalles was the result of deliberate *S. typhimurium* contamination of salad bars in multiple restaurants by residents of Rajneeshpuram.[13] Clandestine laboratories in Rajneeshpuram were used to prepare cultures of *S. typhimurium* that were poured on food items on salad bars and, in some restaurants, into coffee creamers. Commune members said they were testing a plan to incapacitate voters in preparation for an upcoming election. They intended to make citizens of

The Dalles sick on election day to prevent them from voting and thus influence the outcome of the election. The information obtained from informant testimony was incomplete or insufficiently precise to allow direct comparison of dates of contamination with dates of exposure for case customers and case employees on a restaurant-by-restaurant basis. It is likely that some salad bars were contaminated more than once. Informant testimony did indicate that other restaurants, in addition to the 10 identified as group 1 restaurants, might have been targets and that other foods were deliberately contaminated. In addition, produce in at least 1 supermarket was contaminated with *S. typhimurium* and plans were made to contaminate city water.[13]

Oregon State and FBI investigators confiscated an open vial containing a standard strain of *S. typhimurium* (American Type Culture Collection 14028, Rockville, MD) from the clinic laboratory in Rajneeshpuram. Clinic records indicated that the laboratory had obtained this vial from a commercial supplier of biologic products before the outbreak. The *S. typhimurium* strain was indistinguishable from the outbreak strain by antibiogram, biochemical markers, plasmid profiles, and restriction endonuclease digestion of plasmid DNA.

On March 19, 1986, 2 commune members were indicted for conspiring to tamper with consumer products by poisoning food in violation of the federal antitampering act.[12,13] In April 1986, the defendants pleaded guilty to the charges, and in July 1986 they were sentenced to 4 ½ years in prison, to serve concurrently with other sentences.[14]

Using Fault Tree Analysis to Assess Bioterrorist Risks to the U.S. Food Supply
Author: Hope (2004)
DOI: 10.1080/10807030490438382

Hazard identification estimates which bioagent a bioterrorist is most likely to choose, considering the bioterrorist's deployment capabilities, the bioagent's hazard potential (from *Hazard Characterization*) relative to that desired by the bioterrorist, and the target's susceptibility to a given agent. Selection of a bioagent emerges from trade-offs between: (a) a bioa- gent's scientific and technical requirements for safe acquisition (*i.e.*, production, isolation, culturing), management (*i.e.*, handling, storage), and deployment (dissemination) versus a bioterrorist's scientific and technical capabilities in these areas; (b) the logistical, financial, intelligence resource requirements needed to acquire the bioagent and access a target versus a bioterrorist's resources in these areas; and (c) the adverse outcome desired by the bioterrorist (as a function of their objective) versus a bioagent's hazard characteristics,

$$HC = (HZ - HN + 1) \cdot 0.5 \quad (1)$$
$$BC = (BA - BR + 1) \cdot 0.5 \quad (2)$$
$$CC = (CA - CR + 1) \cdot 0.5 \quad (3)$$
$$CB = HC \cdot BC \cdot CC \quad (4)$$

where:

SALMONELLA

HC Bioagent choice based on bioterrorist's objectives (0–1).
HZ Bioagent's hazard potential (0–1); from Hazard Characterization.
HN Bioterrorist's hazard requirements (0–1).
BC Bioterrorist's technical ability to manage the bioagent (0–1).
BA Bioterrorist's scientific and technical capabilities (0–1).
BR Scientific and technical requirements for managing the bioagent (0–1).
CC Bioterrorist's resources to manage the bioagent (0–1).
CA Bioterrorist's financial/logistical resource capabilities (0–1).
CR Financial/logistical resources required to manage the bioagent (0–1).
CB Probability of bioagent being chosen (0–1).

For the Rajneeshee incident, where the bioterrorist's goal was disruption of the local election process and not infliction of mass casualties, a generally non-lethal, but potentially incapacitating bioagent (*Salmonella typhimurium*), was an appropriate choice. The bioterrorist's possessed microbiology laboratory facilities and a scientifically trained staff, as well as access to type cultures of the bioagent, so BA and CA were assumed to be very high (0.95). The bioagent is not extremely difficult to obtain, handle, or maintain, so BR and CR were assumed to be low (0.10), making BC and CC = 0.93. Because the goal was moderate political disruption, bioterrorist's hazard needs (HN) were assumed to be low (0.10). The hazard potential (HZ) of *S. typhimurium* has been estimated as 0.30 (Wade 2001), so HC = 0.60 and there is an average probability (CB = 0.51) of choosing this bioagent.

Exposure assessment describes the pathways a bioagent is expected to follow from its release point to the target and attempts to quantify the amount of bioagent reaching the target. The U.S. food system can be conceptualized as a set of interconnected nodes (**Figure 2**), where farm inputs give rise to animal and crop products, which then move through various interconnected processing and distribution nodes be- fore reaching a human consumer. Changes in agricultural practices have led to more shared nodes (*e.g.*, ≈75% of the U.S. beef stock now passes through just 2% of the nation's feedlots) and geographic concentration of certain nodes (*e.g.*, 84% of U.S. cattle production is in the southwest) (Ban 2000). Dissemination at a concentration node (*e.g.*, large feedlot, grain storage silo, transportation hub, regional food processing facility) could cause a bioagent's appearance in any food distribution networks emanating from this node. A bioagent release (dissemination) could occur at any node. It is assumed that, once released, a bioagent is carried in or on a food item from release point to target, controlled by node interconnections and each node's environmental conditions. If each node is assumed to have the same logical structure (although with potentially different parameter values), fault tree analysis (FTA) can be used within any given node to estimate the probability of the undesired event (Figure 3). By allowing for inputs from prior nodes, it can also be used to evaluate risk at a node several nodes removed from a bioagent's release point. Target exposure is dependent on there being a source either outside or inside that node, in a prior node, or from an intentional release (*i.e.*, bioterrorist act). Several system feedback loops (*e.g.*, animal protein from ingredient processing (slaughtering) used by farm product assemblers (feed lots) that could create re-contamination opportunities are not shown in Figure 2.

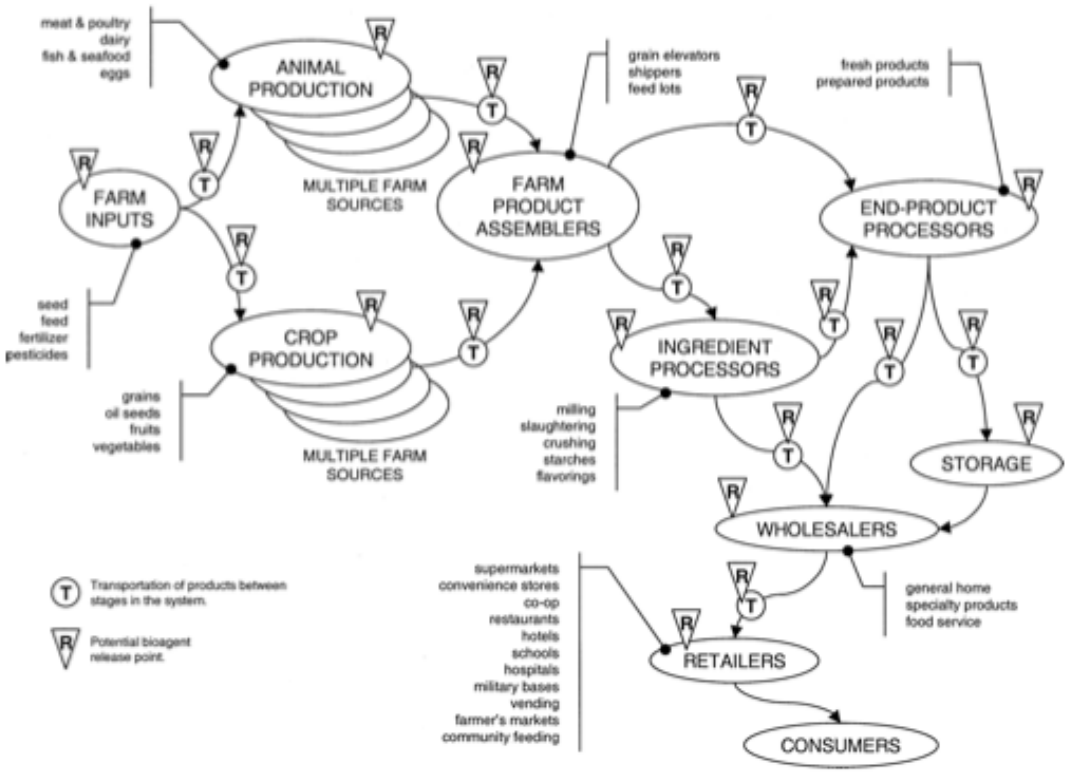


Figure 2. A schematic representation of the U.S. food supply, processing and distribution system, showing flow of agricultural products from supply (production) nodes through various processing nodes to the consumer. Triangles (R) denote potential bioagent release points, while circles (T) indicate transport activity.

SALMONELLA

IMMUNOLOGIST

***Salmonella enterica* serovars Typhimurium and Typhi as model organisms Revealing paradigm of host-pathogen interactions**

Author: Garai et al. (2012)
DOI: 10.4161/viru.21087

Salmonella represents a group of Gram-negative facultative anaerobic pathogenic bacteria which costs millions of lives across the world every year. At present, the genus *Salmonella* is categorized into two species *S. bongori* and *S. enterica*, based on the high (96–99%) sequence similarity of the genome. There is only one subspecies under *S. bongori* namely subspecies V, whereas *S. enterica* comprises the remaining seven subspecies I, II, IIIa, IIIb, IV, VI and VII.[1] Where subspecies I is specific to warm-blooded animals like mammals, others can infect only cold-blooded animals including reptiles. Further division into serovars increases the number of variants to more than 2,500. Out of these, *Salmonella enterica* serovar Typhimurium and *Salmonella enterica* serovar Typhi have been discussed here, as they have previously served as tools to study host-pathogen interactions.

Intracellular pathogens can either survive in a self-constructed niche in the form of a vacuole or they may choose to live in the cytoplasm of the host cell. *Salmonella* chooses the most commonly preferred option of forming an intracellular vacuole termed as *Salmonella* containing vacuole (SCV). SCV arrests the host endosomal pathway at the late endosome stage. … Later the SCV gets juxtaposed to the nucleus by utilizing the microtubule meshwork of the host cell and derives nutrition from the Golgi apparatus.[3]

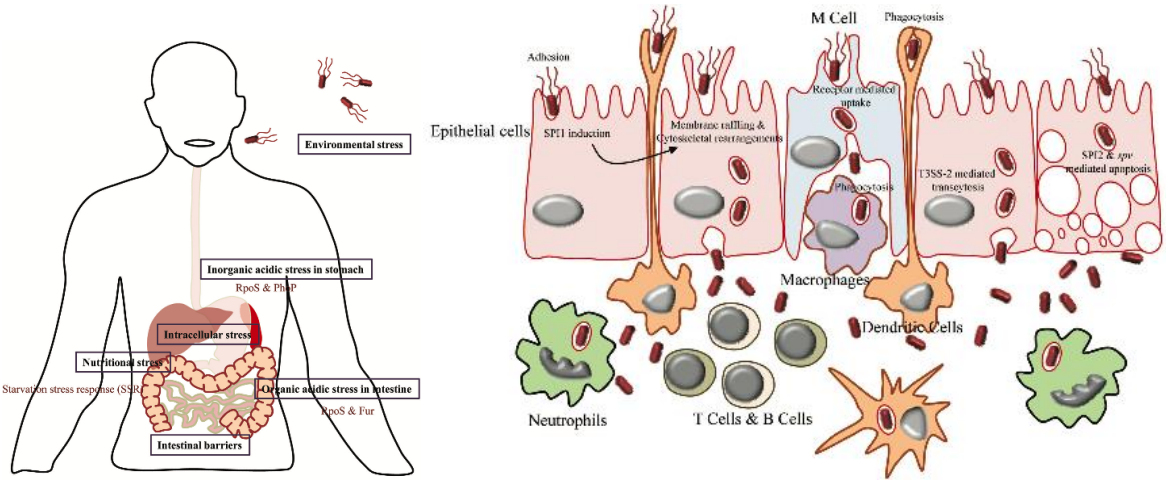


Figure 1. (Left) Challenges encountered by *Salmonella*. The text boxes represent the various stresses encountered by *Salmonella* during its life cycle and the open text describes the factors and signals generated by *Salmonella* in order to combat these stress conditions. **Figure 2. (Right)** Breaching of gut epithelia by *Salmonella*. The mode of entry of *Salmonella* in gut lumen varies according to type of cell encountered on the gut epithelium. The M cells take up the bacteria by means of receptor mediated endocytosis, whereas dendritic cells engulf them by phagocytosis. The membrane of epithelial cells is modified by the action of SPI-1 to facilitate the entry of bacteria. Once inside the gut lumen, *Salmonella* is being taken up by macrophages, T cells, B cells, neutrophils, etc.

SALMONELLA

Mucosal inflammatory response to *Salmonella typhimurium* infection

Author: Patel & McCormick (2014)
DOI: 10.3389/fimmu.2014.00311

Salmonella enterica serovar Typhimurium (*S. typhimurium*) is a Gram-negative, facultative, intracellular anaerobe that causes severe inflammation of the intestinal mucosal epithelium resulting in gastroenteritis. *S. typhimurium* causes disease through its primary virulence mechanism, the type III secretion system (T3SS). There are two T3SSs that are encoded by two regions of the bacterial chromosome called *Salmonella* pathogenicity island 1 and *Salmonella* pathogenicity island 2 (SPI-1 and SPI-2). These pathogenicity islands also encode effector proteins that are secreted from the T3SS and translocated into epithelial cells at the mucosal surface of the intestine.

The architecture of the mucosal epithelium contains several barriers that attempt to prevent or impede infection by pathogenic bacteria. Mechanisms of protection are employed by all of these barriers in order to maintain the integrity of the epithelial cell monolayer and limit inflammation-associated damage (**Figure 1**). *S. typhimurium* can modulate the signaling pathways that govern these mechanisms, including targeting specific proteins or inducing pathways through functional mimicry, in order to provide itself with an ecological advantage with its T3SS virulence mechanism. Although *S. typhimurium* can, in certain instances, bypass the innate immune response, the adaptive inflammatory immune response is in most instances capable of clearing the pathogen, albeit with increased damage to the mucosal epithelium.

MUCUS/MUCINS

The luminal side of the intestinal epithelium is covered with a thick layer of mucus primarily composed of mucins, the main secretory product of goblet cells. Mucins are high molecular weight glycoproteins that aggregate to form a "gel-like" barrier to defend against ... insults. … *S. typhimurium* does not enzymatically degrade mucus in order to colonize the mucosal epithelium. Rather, mucins have actually been shown to be the binding sites for *S. typhimurium*, and in particular a 250-kDa neutral mucin has been implicated as a receptor for *S. typhimurium*. [(9)]

RESIDENT MICROBIOTA

The mammalian intestinal microflora contains ~1014 resident bacteria, comprising ~1,000 species, and they reside in the outer sublayer of the mucosal barrier on the luminal side of the intestinal epithelium (**Figure 1**). The resident microbiota promote resistance to infection by pathogenic microorganisms in several ways. First, they serve as a microbial barrier by competing with pathogens for resources at the outer mucosal sublayer, thereby limiting pathogenic bacterial colonization [(8)]. Additionally, end products of metabolic pathways of individual species of bacteria have been shown to prevent pathogenic infection. … Inflammation provides *S. typhimurium* with a respiratory electron acceptor that members of the resident microbiota are unable to utilize. In particular, reactive oxygen species generated by neutrophils (**PMNs**) during inflammation can react with endogenous thiosulfate to form tetrathionate unnder anaerobic conditions in which thiosulphate was oxidized to tetrathionate, *S. typhimurium* displays a growth advantage in comparison to resident microbiota under the same conditions.

SALMONELLA

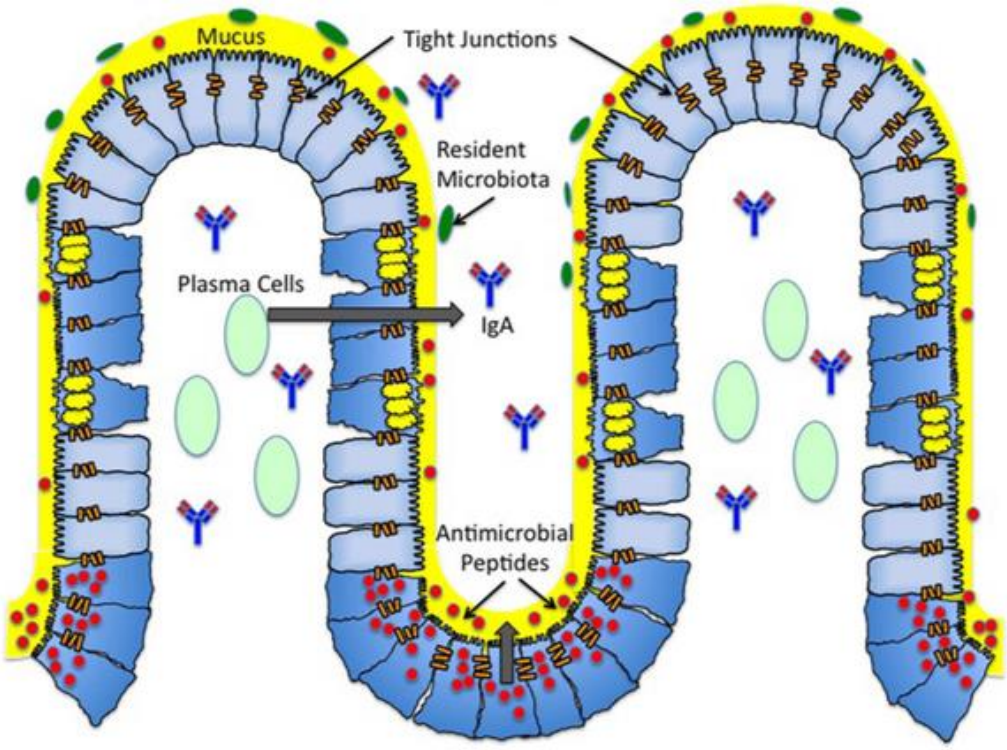


FIGURE 1 | Architecture of the mucosal surface. The mucosal surface of the intestine contains a single layer of epithelial cells. The monolayer of epithelial cells is fortified by a layer of mucus (yellow) produced by *Goblet cells* (*blue cells with yellow granules*). This thick mucus layer contains membrane bound and secreted mucins. The antimicrobial peptides (red) secreted by *Paneth cells* (*blue cells with red granules*) reside in the thick mucus layer, providing another form of protection against both pathogenic and commensal bacteria. Antimicrobial peptides include defensins, cathelicidins, and histatins. *Plasma B cells* (*light green*) reside in the subepithelial region and produce *secretory IgA* (*blue and red antibody*). Secreted IgA is found in the subepithelial region and the lumen. *Resident microbiota* (*green*) reside in the outer mucus layer, providing yet another barrier to pathogenic infection. The seal between epithelial cells is maintained by *tight junctions* (*orange bars*).

THE TYPE III SECRETION SYSTEM (T3SS)

Upon contact *of S. typhimurium* with the epithelial cell monolayer, the [T3SS system releases] effector proteins … activating host cell GTPases resulting in actin rearrangements. *S. typhimurium* is engulfed by epithelial cells through a macropinocytosis event termed bacterial-mediated endocytosis, and is ultimately contained within in a membrane-bound vesicle called a *Samonella* containing vacuole (SCV). *Salmonella typhimurium* also targets antigen-sampling microfold (M) cells to translocate across the gut epithelium. *S. typhimurium* type III effector protein also induces an epithelial–mesenchymal transition of the follicle-associated epithelium cells into M cells. After successful entry into epithelial cells and restoration of the epithelial cell membrane, *S. typhimurium* relies primarily on the T3SS encoded by SPI-2 to survive and replicate intracellularly. *S. typhimurium* T3SS also produces encoding factors that mediate the evasion of immune responses by … promoting protection from reactive oxygen intermediates produced by macrophages, specifically nitric oxide (NO) and NADPH oxidase.

NEUTROPHIL RECRUITMENT

A hallmark of *S. typhimurium*-induced inflammation is the recruitment of PMNs from the underlying microvasculature to the subepithelial region of the epithelial cell monolayer (**Figure 2**). The neutrophils then migrate across the monolayer into the lumen, resulting in the inflammatory pathology of Salmonellosis. it is becoming increasingly appreciated how inflammation induced by *S. typhimurium* increases its pathogenic bacterial fitness.

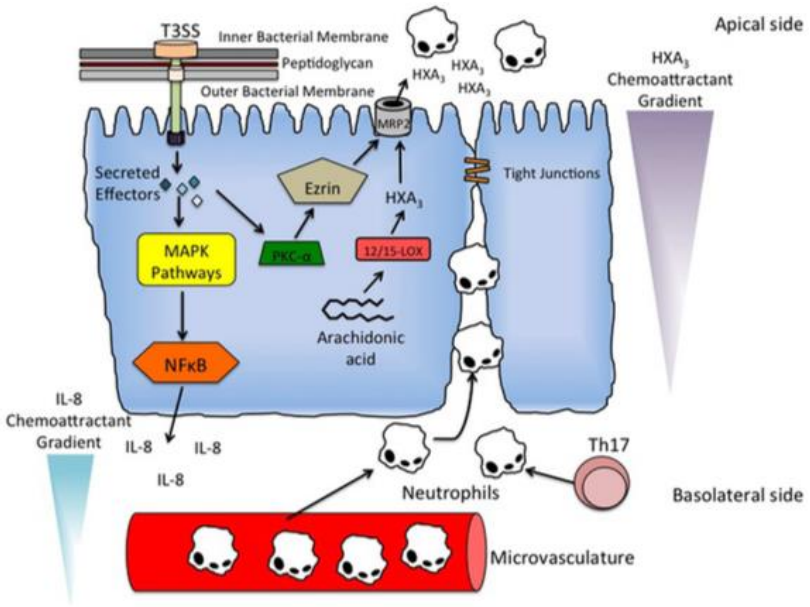


FIGURE 2 | Mechanism of PMN recruitment and PMN transmigration

S. typhimurium utilizes its T3SS to secrete effector proteins into epithelial cells to activate inflammatory signaling pathways. In particular, the activation of Rho-GTPases by SopE, SopE2, and SopB result in the induction of mitogen-activated protein kinase (MAPK) pathways. Activation of NF-κB results in … producing an IL-8 chemoattractant gradient that recruits neutrophils to the subepithelial region from the underlying microvasculature. Th17 cells are also present in the subepithelial region, and function to recruit and activate neutrophils in the subepithelium. PMN transmigration is facilitated by another chemoattractant HXA^3.

HISTORICAL CONTEXT

Pathogens in focus, *Vibrio cholerae*: Cholera toxin

Author: Vanden Broeck et al. (2007)
DOI: 10.1016/j.biocel.2007.07.005

Vibrio cholerae is a major pathogen responsible for the life-threatening acute diarrhoea, cholera, which mainly affects third world populations. *V. cholerae* are Gram-negative bacteria which belong to the *Vibrionaceae* family. Despite rehydration therapy with uncontaminated water and electrolytes, *V. cholerae* infection still results in high morbidity and mortality rates. In regions where sanitary provisions are poor, cholera is often endemic, and symptomless carriers usually induce epidemic outbreaks mostly among people with an impaired immune system such as young children, the elderly or travellers. Historically, seven pandemic outbreaks have been described, of which the seventh is still ongoing in South-Asia and Bangladesh with over 1million cases and more than 10,000 deaths (WHO, 2006).

The epidemic spread of cholera was first recognized by John Snow in 1854 during the fourth pandemic and in the same year, the bacteria was described and termed *V. cholerae* by Filippo Pacini. The discovery of the causative protein factor, cholera toxin (CT), was first suggested by Robert Koch in 1884 and demonstrated 75 years later (Finkelstein, Mukerjee, & Rudra, 1963).

Cholera

Author: Sack et al. (2004)
DOI: 10.1016/S0140-6736(03)15328-7

Intestinal infection with *Vibrio cholerae* results in the loss of large volumes of watery stool, leading to severe and rapidly progressing dehydration and shock. Without adequate and appropriate rehydration therapy, severe cholera kills about half of affected individuals. Cholera toxin, a potent stimulator of adenylate cyclase, causes the intestine to secrete watery fluid rich in sodium, bicarbonate, and potassium, in volumes far exceeding the intestinal absorptive capacity. Cholera has spread from the Indian subcontinent where it is endemic to involve nearly the whole world seven times during the past 185 years. *V. cholera* serogroup O1, biotype El Tor, has moved from Asia to cause pandemic disease in Africa and South America during the past 35 years. A new serogroup, O139, appeared in south Asia in 1992, has become endemic there, and threatens to start the next pandemic. Research on case management of cholera led to the development of rehydration therapy for dehydrating diarrhoea in general, including the proper use of intravenous and oral rehydration solutions. Appropriate case management has reduced deaths from diarrhoeal disease by an estimated 3 million per year compared with 20 years ago. Vaccination was thought to have no role for cholera, but new oral vaccines are showing great promise.

“Asiatic cholera”, as it was sometimes called, has been endemic in south Asia, especially the Ganges delta region, from the time of recorded history. It was always much feared because it regularly occurred in epidemics with high mortality rates. In Kolkata, a cholera temple, Ola [Bibi] (“our lady of the flux”), was built for protection against the disease. In 1817, the first

cholera pandemic began with spread of the disease outside the Indian subcontinent along trade routes to the west as far as southern Russia. A second pandemic started in 1826 and reached the major European cities by the early 1830s. In 1831, the pandemic reached the UK and the response was important in that it led to the establishment of local Boards of Health and a "Cholera Gazette", which served as a clearing house for tracking the epidemic.[3]

At that time cholera was thought to be spread by the "miasma" (like a fog) coming from the river, but the classic epidemiological study of John Snow in 1854 in London showed the association of the disease with contaminated drinking water even before any bacteria were known to exist.[4] Three more pandemics, continuing up to 1925, involved Africa, Australia, Europe, and all the Americas. The causative agent, *Vibrio cholerae*, was not identified until 1884 in Kolkata during the fifth pandemic.[5] Why the earlier pandemics began and how they ended is not known. However, cholera did not persist in any of the new geographical areas that it had invaded but continued as an endemic disease in the Ganges delta.

Because of the large numbers of cases and deaths during these pandemics, the disease was viewed as a major public-health disaster requiring governmental intervention. The New York cholera epidemic led to the first Board of Health in the USA in 1866,[6] and cholera became the first reportable disease.

Cholera transmission: the host, pathogen and bacteriophage dynamic
Author: Nelson et al. (2009)
DOI: 10.1038/nrmicro2204

Diarrhoeal diseases, including cholera, are the leading cause of morbidity and the second most common cause of death among children under 5 years of age globally[1,2]. It is difficult to gauge the exact morbidity and mortality of cholera because the surveillance systems in many developing countries are rudimentary, and many countries are hesitant to report cholera cases to the WHO because of the potential negative economic impact of the disease on trade and tourism. Today, the true burden of cholera is estimated to reach several million cases per year, predominantly in Asia and Africa[3]. With optimal delivery, oral rehydration therapy can lower case fatality rates from the >20% seen historically[4–6] to <1%[7]. Much work remains to be done, as 27 countries reported case fatality rates above the 1% threshold in 2007 (REF. 8).

Cholera: Lessons from Haiti and Beyond
Author: Weil et al. 2012)
DOI: 10.1007/s11908-011-0221-9

Notably, the island of Hispaniola (Haiti and the Dominican Republic) was spared the 1991 cholera epidemic that affected much of South and Central America, and there are no microbiologically confirmed records of cholera infection occurring in Haiti before 2010. On October 20 2010, 10 months after the January 12 earthquake, Haitian authorities reported an increase in acute severe diarrhea. Within days, the National Public Health Laboratory in Haiti isolated *V. cholerae* serogroup O1, serotype Ogawa. Less than 1 month later, cholera cases

emerged in all ten departments in Haiti and nearly 1000 deaths were reported. As of August 29, 2011, over 6200 deaths and more than 439,000 cases [Population 10.71 million as of 2015] have been reported by the Haitian Ministry of Health. Cholera will likely remain in Haiti for many years to come.

In 2008, 30% of persons in Haiti had no access to a latrine or toilet, and only 17% had access to adequate sanitation. Systemic poverty, a strained health care system, and poor sanitation allowed cholera to spread rapidly. At early stages of the cholera epidemic, a lack of knowledge about cholera and transportation challenges led to deaths at home before patients could reach care. Initial treatment centers, lacking healthcare personnel, supplies and infection control mechanisms experienced high case fatality rates (CFR) of around 6%. Early efforts at outbreak control focused on education, distribution of potable water and water purification tablets, promoting appropriate rehydration at a community level, and spreading prevention messages including the need for water treatment and appropriate disposal of fecal material. As national and international resources were mobilized, materials and supplies were provided and appropriate infection control mechanisms were established in cholera treatment centers. ORS was widely distributed and healthcare workers gained experience treating cholera, and the CFR in cholera treatment centers decreased.

MOLECULAR BIOLOGIST

Pathogens in focus, *Vibrio cholerae*: Cholera toxin
Author: Vanden Broeck et al. (2007)
DOI: 10.1016/j.biocel.2007.07.005

The *V. cholerae* genome comprises two chromosomes, of which chromosome I harbours all virulence factors, including CT and the toxin-co-regulated pilus (TCP). *V. cholerae* colonises the small bowel using TCP and interacts with receptors on the intestinal epithelium. Once attached, the bacterium secretes its toxin which is accompanied by the release of hemagglutinin/protease (HA/protease). This extracellular HA/protease is responsible for nicking the CT-A subunit at Arg192, yielding discrete CT-A1 and CT-A2 subunits which are solely connected by a single disulfide bond. This post-translational modification is critical for full activity of the toxin, leading to an increase in cAMP-production.

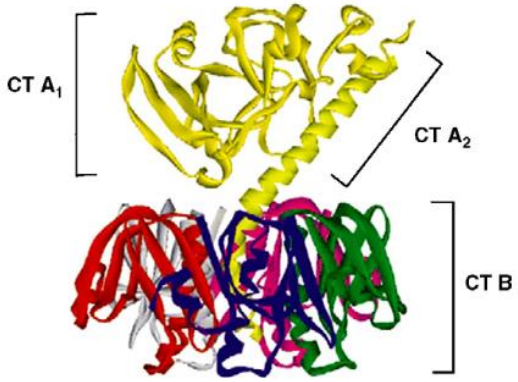


Figure 1. CT structure. Crystal structure of cholera toxin. The heterodimeric CT-A subunit (depicted in yellow) consists of two polypeptide chains, CT-A_1 and CT-A_2, linked by a single disulfide bond. CT-A_1 is enzymatically active and displaced mono-ADP-ribosyltransferase activity while CT-A_2 functions as a linker between CT-A_1 and CT-B. The B subunit is comprised of five identical polypeptide chains (illustrated in green, purple, red, green and blue), each with GM1 binding capacity.

CT belongs to the superfamily of AB toxins and is an oligomeric protein composed of a heterodimeric A-subunit and a homopentameric B-subunit (Fig. 1). The five identical B monomers are arranged in a ring-like configuration with each a single binding site for the plasma membrane receptor of the jejunal intestinal epithelial cells. The CT-A subunit consists of two distinct polypeptide chains linked by a single disulfide bridge. The CT-A2 polypeptide has a linker function and occupies the central channel and goes through the doughnut-like structure of the CT-B pentamer, tethering CT-A1 and CT-B subunits. Additionally, based on the primary sequence of the CT- gene, the CT-A2 subunit possesses a C-terminal KDEL (Lys/Asp/Glu/Leu) retrieval signal, inferred to play a role in retrograde trafficking from Golgi to the endoplasmic reticulum (ER). CT-A1 is a catalytic polypeptide displaying mono-ADP-ribosyltransferase activity, involved in ADP-ribosylation of the G_s -subunit of a stimulatory GTP-binding regulatory protein, followed by stimulation of basolateral adenylate cyclase (AC).

CHOLERA

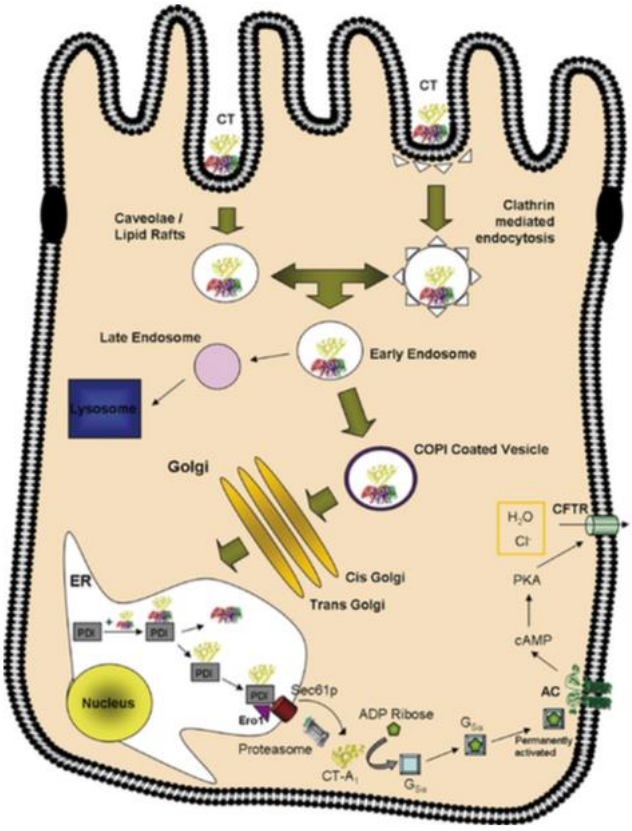


Figure 2. Proposed model for the intoxication of the enterocyte by CT. CT binds with high affinity its receptor GM1, after which it is actively internalized by the host cell machinery into the early and recycling endosomes, regardless of the mechanism of internalization. Subsequently, CT is transported as a holotoxin in a retrograde manner to the Golgi and further to the ER. Here, CT dissociates into a CT-A1 and a CT-A2/CT-B complex driven by PDI, which is also responsible for the unfolding of CT-A1. CT-A1 is further retrotranslocated via the Sec61p complex to the cytosol, avoiding proteasomal degradation, where it associates with basolateral AC. Transfer of mono-ADP-ribose from NAD+ to GS constitutively triggers the adenylate cyclase (AC), resulting in a substantial increase in intracellular concentrations of cAMP, followed by a protein kinase (PKA)-mediated phosphorylation of the major chloride channel of intestinal epithelial cells, the cystic fibrosis transmembrane conductance regulator (CFTR). The net increase in Cl^--secretion is accompanied by osmotic movement of a large quantity of water into the intestinal lumen, resulting in severe diarrhea.

A Therapeutic Chemical Chaperone Inhibits Cholera Intoxication and Unfolding/Translocation of the Cholera Toxin A1 Subunit

Author: Taylor et al. (2011)*

DOI: 10.1371/journal.pone.0018825

*Research carried out at UCF.

Introduction

AB toxins consist of an enzymatic A subunit and a cell-binding B subunit [1]. These toxins are secreted into the extracellular milieu, but they act upon targets within the eukaryotic cytosol. The

toxins must therefore cross a membrane barrier in order to function. Some AB toxins travel by vesicle carriers from the cell surface to the endoplasmic reticulum (ER) before passing into the cytosol [2]. These ER-translocating toxins enter the ER as intact holotoxins, but environmental conditions in the ER promote the dissociation of the catalytic subunit from the rest of the toxin. Translocation of the isolated A chain from the ER to the cytosol is then facilitated by the quality control mechanism of ER-associated degradation (ERAD) [3]. Exported ERAD substrates are normally targeted for ubiqutin-dependent proteasomal degradation, but the A chains of ER-translocating toxins have few lysine residues for ubiquitin conjugation and thus effectively avoid degradation by the 26S proteasome [4–7].

Characterization of XerC- and XerD-dependent CTX phage integration in Vibrio cholera
Author: McLeod & Waldor (2004)
DOI: 10.1111/j.1365-2958.2004.04309.x

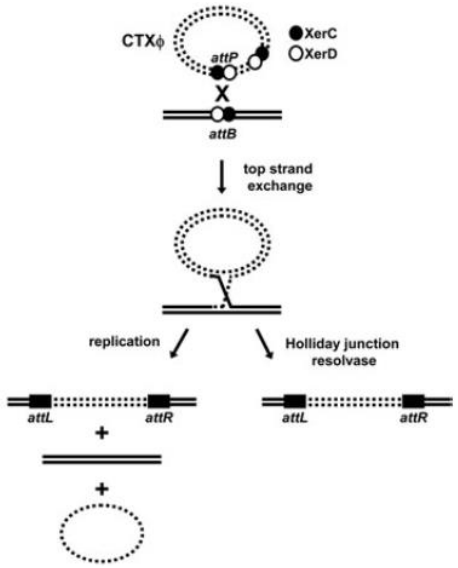


Figure 8. Model of CTXf integration by XerC and XerD into the *dif* region of *V. cholerae* chromosome I. Site-specific recombination between the phage attachment region (*attP*) and the bacterial attachment site (*attB*) generates the left and right phage–chromosome junctions (*attL* and *attR*). The double-stranded form of the phage genome is shown with the dotted lines. The bacterial chromosome is shown as a solid double line. XerC, depicted as filled circles, and XerD, depicted as unfilled circles, bind in close proximity to each other to one region on *attB* and to two sites on *attP*. The XerC/D binding sites are in inverted orientations to one another within *attP*. The proposed mechanism of integrative recombination involves cleavage and exchange of the top DNA strands by the XerC/D complex to form a four-way junction intermediate. Subsequent resolution of this structure could occur by the activity of a host **Holliday junction** resolvase or the passage of the replication fork.

Holliday Junction: Cross-shaped structure that forms during the process of genetic recombination when two double-stranded DNA molecules become separated into four strands in order to exchange information.

EVOLUTIONARY BIOLOGIST

Cholera

Author: Sack et al. (2004)
DOI: 10.1016/S0140-6736(03)15328-7

The current (seventh) pandemic now has involved almost the whole world. This pandemic began in Indonesia,[7] rather than the Ganges delta, and the causative agent was a biotype of *V. cholerae* serogroup O1 called El Tor. It was first isolated in 1905 from Indonesian pilgrims travelling to Mecca at a quarantine station in the village of El Tor, Egypt.[2] It was found again in 1937 in Sulawesi, Indonesia.[8] Then in 1960, for unknown reasons, this strain began to spread around the world. It invaded India in 1964, Africa in 1970,[9–11] southern Europe in 1970,[12,13] and South America in 1991.[14,15] The disease has now become endemic in many of these places, particularly south Asia and Africa. Since 1973, a focus of El Tor *V. cholerae* similar but not identical to the pandemic strain has persisted in the Gulf of Mexico of the USA causing sporadic cases of summertime, seafood-associated cholera.[16]

In 1992, a newly described, non-O1 serogroup of *V. cholerae*, designated O139 Bengal, caused unusual cholera outbreaks in India and Bangladesh.[17,18] Before the discovery of *V. cholerae* O139 (the 139th serotype in the typing scheme for *V. cholerae)*, only serogroup O1 was known to cause epidemic cholera, so the O139 serotype was essentially a "new" cause of cholera.[19] Serogroups O139 Bengal and O1 now coexist and continue to cause large outbreaks of cholera in India and Bangladesh. The O139 serogroup is likely to be the cause of the next (eighth) pandemic of cholera.

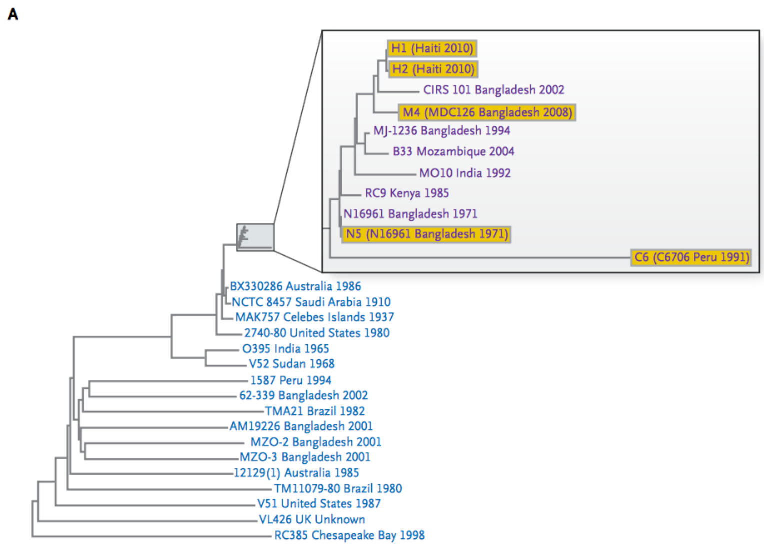
The Origin of the Haitian Cholera Outbreak Strain

Author: Chin et al. (2011)
DOI: 10.1056/NEJMoa1012928

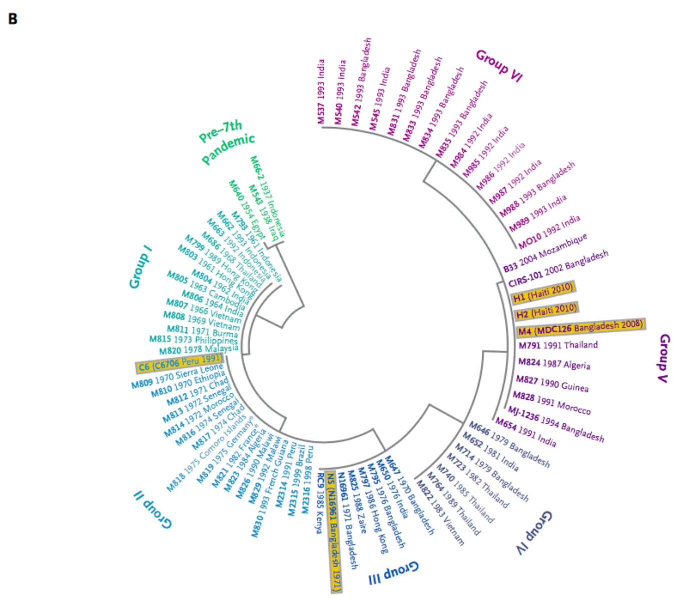
Introduction
Traditionally, *V. cholerae* strains are classified into serogroups on the basis of the structure of an outer-membrane O antigen and into biotypes on the basis of a variety of biochemical and microbiologic tests. The ongoing seventh pandemic of cholera is caused by the *V. cholerae* El Tor biotype of serogroup O1 (El Tor O1),[5] which has replaced the previous "classical" biotype and has spread globally since its appearance in Indonesia in 1961. It reached the Americas in 1991, beginning in Peru and then spreading throughout much of South America and Central America, where it has since become endemic[6]; however, the strains of *V. cholerae* El Tor O1 that are now endemic in South America and Central America had not previously been reported to have caused cholera on Hispaniola. Analyses carried out by Haitian and U.S. laboratories have indicated that the current outbreak strain in Haiti is also *V. cholerae* El Tor O1 and thus is related to strains that are causing the ongoing seventh pandemic of cholera.

Both genetic and phenotypic diversity have arisen among circulating strains of *V. cholerae* El Tor O1, reflecting the acquisition, loss, or alteration of mobile genetic elements, including CTX phage, which bears the genes encoding cholera toxin[7]; genomic islands[8]; and SXT-family

integrative and conjugative elements, which often encode resistance to several antibiotics.[9] Single-nucleotide variations (SNVs) and insertions and deletions have also been detected in the core *V. cholerae* genome.[10,11] Such heterogeneity has been used to group strains and to model and understand their transmission around the globe[10,11] and is most comprehensively captured by sequencing genomic DNA.



Panel A shows the phylogenetic relationships among pandemic *V. cholerae* strains on the basis of single-nucleotide variations (SNVs) identified among all strains for which a set of 1588 orthologous genes has been completely sequenced.[10] The magnified inset represents strains in the seventh pandemic, including H1, H2, M4, C6, and N5. Although the Haitian strains are similar to isolates from Latin America (C6 from the 1991 outbreak in Peru) and Africa (B33 from the 2004 outbreak in Mozambique), they are most closely related to recent South Asian isolates (M4 from the 2008 outbreak in Bangladesh and CIRS101 from the 2002 outbreak in Bangladesh)



Panel B shows the phylogenetic relationships among a broad set of seventh-pandemic

V. cholerae strains.[19] The phylogenetic tree is rooted with three pre–seventh-pandemic strains. Six groups from the seventh pandemic are readily identified in this tree, with H1 and H2 falling into group V, which also includes variant strains from Bangladesh (CIRS101 and M4). The phylogeny highlights the distance between strains of group V and those of group II, the latter of which consists mainly of Latin American strains (including C6, the strain we sequenced) and African strains isolated between 1970 and 1998. It supports the conclusion that Haitian *V. cholerae* is more closely related to contemporary South Asian strains of *V. cholerae* than to Latin American strains. The placement of C6 in group II is consistent with a previously proposed hypothesis that Latin American strains of *V. cholerae* may have been introduced from Africa.[19]

Molecular mechanism of acquisition of the cholera toxin genes
Author: Das et al. (2011)

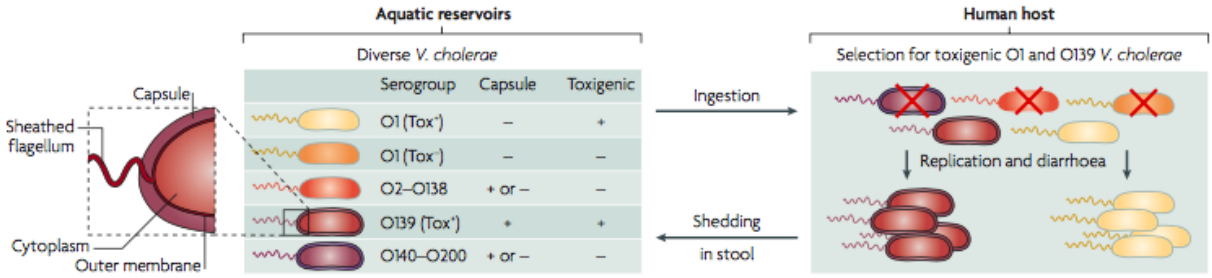
Introduction
Most bacteriophages are detrimental to their host metabolism. However, phages also participate in the horizontal transfer of genes among bacteria because their genome can harbour other genes than those strictly required for their life cycle. This can be highly beneficial to the bacterial host. Indeed, many bacterial virulence factors are associated with phage-like DNA sequences. More strikingly, the exotoxins produced by many pathogenic bacteria are encoded in the genome of lysogenic phages. This is notably the case in *Bordetella avium*[1], *Clostridium botulinum*[2], *Corynebacterium diphtheria*[3], *Escherichia coli*[4], *Pseudomonas aeruginosa*[5], *Shigella dysenteriae*[6], *Staphylococcus aureus*[7] *and Streptococcus pyogenes*[8]. The integrated prophages harboured by these bacteria profit from the multiplication of their host in the environment, which is in turn favoured by the virulence factors they bring to their host.

The study of *Vibrio cholerae*, the agent of the deadly diarrhoeal disease cholera, provides a fascinating case of such a bacterium-phage co-evolution. *V. cholerae* is the host for a variety of phages, commonly known as vibriophages, which can be lytic, non-lytic, virulent or temperate[9]. On the one hand, phage predation of *V. cholerae* has been reported to be a factor that influences seasonal epidemics of cholera[10]. On the other hand, one of the major virulence factors of *V. cholerae*, cholera toxin, is encoded in the genome of an integrated prophage CTXΦ.

Cholera transmission: the host, pathogen and bacteriophage dynamic
Author: Nelson et al. (2009)
DOI: 10.1038/nrmicro2204

Introduction
The causative agent of cholera, the Gram-negative bacterium *Vibrio cholerae*, is a facultative pathogen that has both human and environmental stages in its life cycle[9,10]. *V. cholerae* is differentiated serologically on the basis of the O antigen of its lipopolysaccharide (LPS) (FIG. 1). Cholera toxin-producing (toxigenic) strains of the O1 and O139 serogroups cause the vast majority of the disease. The O1 serogroup is subdivided into two phenotypically distinct biotypes, El Tor and classical, the second of which is associated with earlier pandemics. Both biotypes can be further subdivided into two serotypes, Inaba and Ogawa[7]. In the past 20 years, El

Tor has replaced the classical biotype[11]; however, the legacy of the classical biotype lingers, as El Tor strains harbouring classical cholera toxin have emerged[12–14]. The O139 serogroup first appeared in 1992, as a result of a multi-gene substitution in the O antigen-coding region of a progenitor O1 El Tor strain[15]. Although the O139 serogroup caused devastating outbreaks in the 1990s, the El Tor strain remains the dominant strain globally[11,16,17].



Phylogenetic relationship of *Vibrio cholerae* strains. On the basis of the antigenicity of the O antigen component of the outer membrane lipopolysaccharide, more than 200 serogroups (O1–O200) of *Vibrio cholerae* exist in aquatic environments. Only a subset of O1 and O139 serogroup strains are toxigenic (Tox+) and therefore capable of causing cholera when ingested; such strains are selected for in the host. Other strains are non-toxigenic (Tox–) and are selected against. Different O antigen types are indicated by the colour of the outer membrane and sheathed flagellum (the periplasmic space and the inner membrane are not shown). Capsules are present in a subset of strains. Different strain genotypes are indicated by the colour of the cytoplasm; note that Tox+ O1 and O139 have essentially the same genotype, with the exception of the O antigen genes.

STATISTICIAN

The Origin of the Haitian Cholera Outbreak Strain

Author: Chin et al. (2011)
DOI: 10.1056/NEJMoa1012928

Introduction

The outbreak of cholera that began in Haiti in late October 2010 illustrates the continued public health threat of this ancient scourge.[1] Cholera, an acutely dehydrating diarrheal disease that can rapidly kill its victims, is caused by *Vibrio cholerae,* a gram-negative bacterium.[2] This disease, which is usually transmitted through contaminated water, can and has spread in an explosive fashion. In the weeks since cases were first confirmed in the Artibonite province of Haiti on October 19, 2010, the disease has reached all 10 provinces in Haiti and has spread to the neighboring Dominican Republic on the island of Hispaniola. Of the more than 93,000 persons who have been sickened from the outbreak, more than 2100 have died, according to the Haitian Ministry of Public Health and Population (www.mspp.gouv), and it is thought that the epidemic has not yet peaked.[3] Cholera epidemics had not been reported in Haiti for more than a century, and the origin of the Haitian *V. cholerae* outbreak has been the subject of some controversy.[4]

Discussion

Our findings have policy implications for public health officials who are considering the deployment of vaccines or other measures for controlling cholera.[29,30] The apparent introduction of cholera into Haiti through human activity emphasizes the concept that predicting outbreaks of infectious diseases requires a global rather than a local assessment of risk factors.

The accidental introduction of South Asian variant *V. cholerae* El Tor into Haiti may have consequences beyond Haiti. The apparently higher relative fitness[23,24] and increased antibiotic resistance of the South Asian strains and the ability of those strains to cause severe cholera[23] suggest that the South Asian variant *V. cholerae* El Tor that is now in Haiti could displace the resident El Tor O1 seventh-pandemic strains in Latin America. It is likely that the Caribbean ecosystem may now be host to a set of genes, including classical biotype-like cholera toxin genes and the STX integrative and conjugative element, that were previously absent from this region. Clearly, the provision of adequate sanitation and clean water is essential for preventing the further spread of the Haitian cholera epidemic.[3] Vaccination would also help to prevent the spread of disease, although cholera vaccines are in short supply. Our findings suggest that public health measures to counter the spread of cholera[30-32] in Hispaniola could minimize the dissemination of the new South Asian strain and the virulence genes that it carries beyond the shores of this Caribbean island.

Prediction of the spatial evolution and effects of control measures for the unfolding Haiti cholera outbreak

Author: Bertuzzo et al. (2011)
DOI: 10.1029/2011GL046823

Results

CHOLERA

In our metacommunity framework, Haiti is divided into more than 500 local communities (representing the fourth administrative level, see Figure 1). For each community we model the dynamics of the number of susceptibles to the disease (initially coinciding with the total population, which had never been exposed to cholera and thus was lacking any immunity [Butler, 2010; Chin et al., 2011]), infected individuals and *V. cholerae* abundance in the aquatic environment. The key assumptions are that the rate at which susceptibles become infected depends on the concentration of pathogens in the available water and, in turn, that new free-living bacteria are produced by infected individuals through fecal contamination.

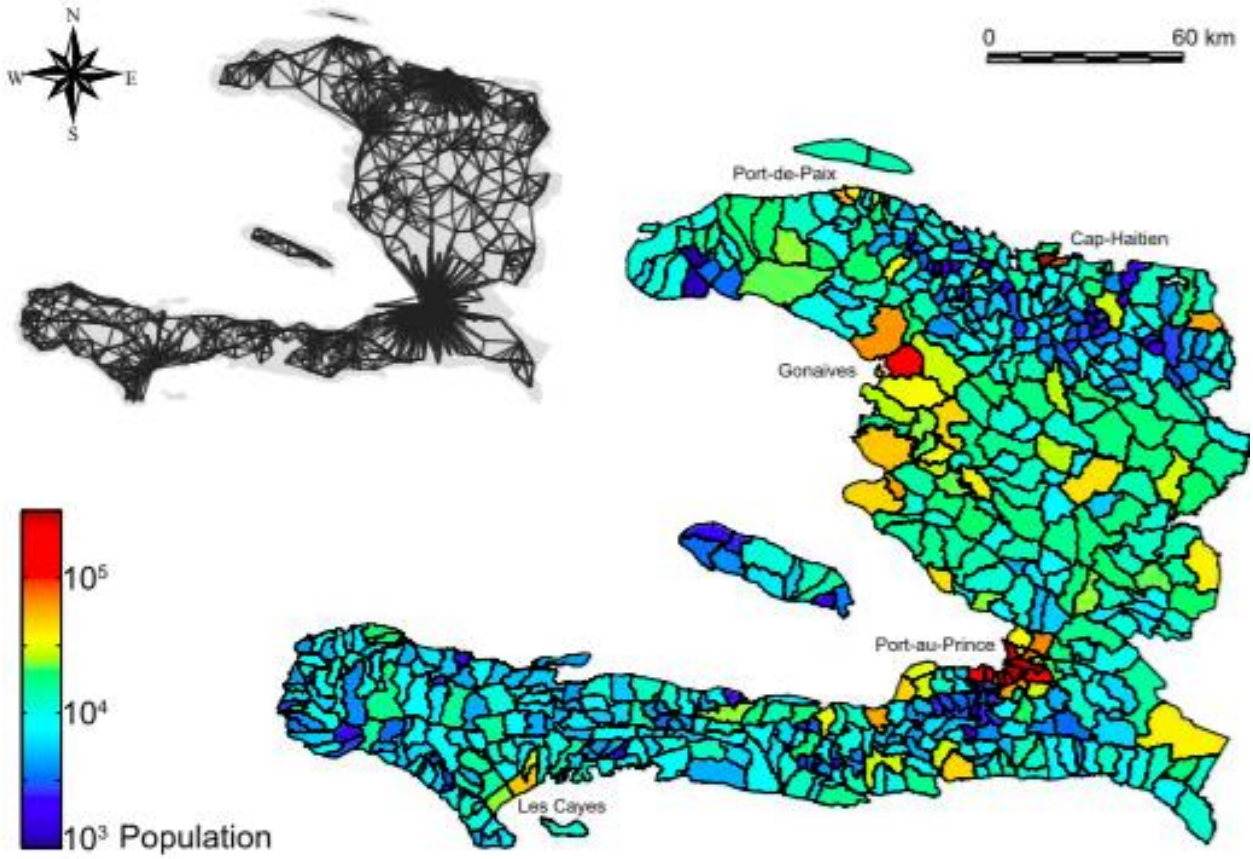


Figure 1. Maps of Haiti. Administrative subdivision (fourth level) color coded accordingly to population size. Each district represents a human local community in the model. The inset shows the network used to model the dispersion of pathogens among communities. For each node only the four most important outbound connections, as computed from the gravity model, are shown.

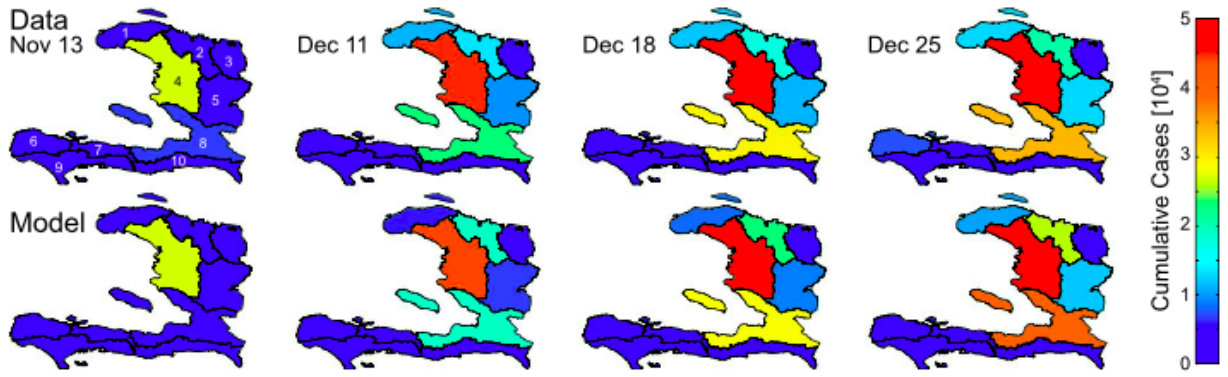


Figure 2. Spatial spreading of the Haiti cholera epidemic. Comparison between data and model results in the ten departments: 1) Nord-Ouest, 2) Nord, 3) Nord-Est, 4) Artibonite, 5) Centre, 6) Grand Anse, 7) Nippes, 8) Ouest, 9) Sud, 10) Sud-Est.

As shown in Figures 2 and 3, the model is able to reproduce the timing and the magnitude of the epidemic in the ten Haitian departments, in particular in Artibonite and Ouest, the most populated – and most scourged by the disease – regions.

U.N. Admits Role in Cholera Epidemic in Haiti
Author: Katz (2016)

For the first time since a cholera epidemic believed to be imported by United Nations peacekeepers began killing thousands of Haitians nearly six years ago, the office of Secretary General Ban Ki-moon has acknowledged that the United Nations played a role in the initial outbreak and that a "significant new set of U.N. actions" will be needed to respond to the crisis.

…

Mr. Alston also argued in his report that, as The New York Times has reported, the United Nations' cholera eradication program has failed. Infection rates have been rising every year in Haiti since 2014, as the organization struggles to raise the $2.27 billion it says is needed to eradicate the disease from member states. No major water or sanitation projects have been completed in Haiti; two pilot wastewater processing plants built there in the wake of the epidemic quickly closed because of a lack of donor funds.

In a separate internal report released days ago after being withheld for nearly a year, United Nations auditors said a quarter of the sites run by the peacekeepers with the organization's Stabilization Mission in Haiti, or Minustah, that they had visited were still discharging their waste into public canals as late as 2014, four years after the epidemic began.

"Victims are living in fear because the disease is still out there," Mario Joseph, a prominent Haitian human rights lawyer representing cholera victims, told demonstrators in Port-au-Prince last month. He added, "If the Nepalese contingent returns to defecate in the water again, they will get the disease again, only worse."

CHOLERA

In 2011, when families of 5,000 Haitian cholera victims petitioned the United Nations for redress, its Office of Legal Affairs simply declared their claims "not receivable." (Mr. Alston called that argument "wholly unconvincing in legal terms.")

Those families and others then sued the United Nations, including Mr. Ban and the former Minustah chief Edmond Mulet, in federal court in New York. (In November, Mr. Ban promoted Mr. Mulet to be his chief of staff.) The United Nations refused to appear in court, claiming diplomatic immunity under its charter, leaving Justice Department lawyers to defend it instead. That case is now pending a decision from the Second Circuit Court of Appeals in New York. The redress demanded by families of the 10,000 people killed and 800,000 affected would reach $40 billion, Mr. Alston wrote — and that figure does not take into account "those certain to die and be infected in the years ahead."

"Since this is almost five times the total annual budget for peacekeeping worldwide, it is a figure that is understandably seen as prohibitive and unrealistic," he said. Still, he argued: "The figure of $40 billion should stand as a warning of the consequences that could follow if national courts become convinced that the abdication policy is not just unconscionable but also legally unjustified. The best way to avoid that happening is for the United Nations to offer an appropriate remedy.

Understanding the cholera epidemic, Haiti
Author: Piarroux et al. (2011)
DOI: 10.3201/eid1707.110059

Our epidemiologic study provides several additional arguments confirming an importation of cholera in Haiti. There was an exact correlation in time and places between the arrival of a Nepalese battalion from an area experiencing a cholera outbreak and the appearance of the first cases in Meille a few days after. The remoteness of Meille in central Haiti and the absence of report of other incomers make it unlikely that a cholera strain might have been brought there another way. DNA fingerprinting of V. cholerae isolates in Haiti [(1)] and genotyping [(7,21)] corroborate our findings because the fingerprinting and genotyping suggest an introduction from a distant source in a single event [(22)].

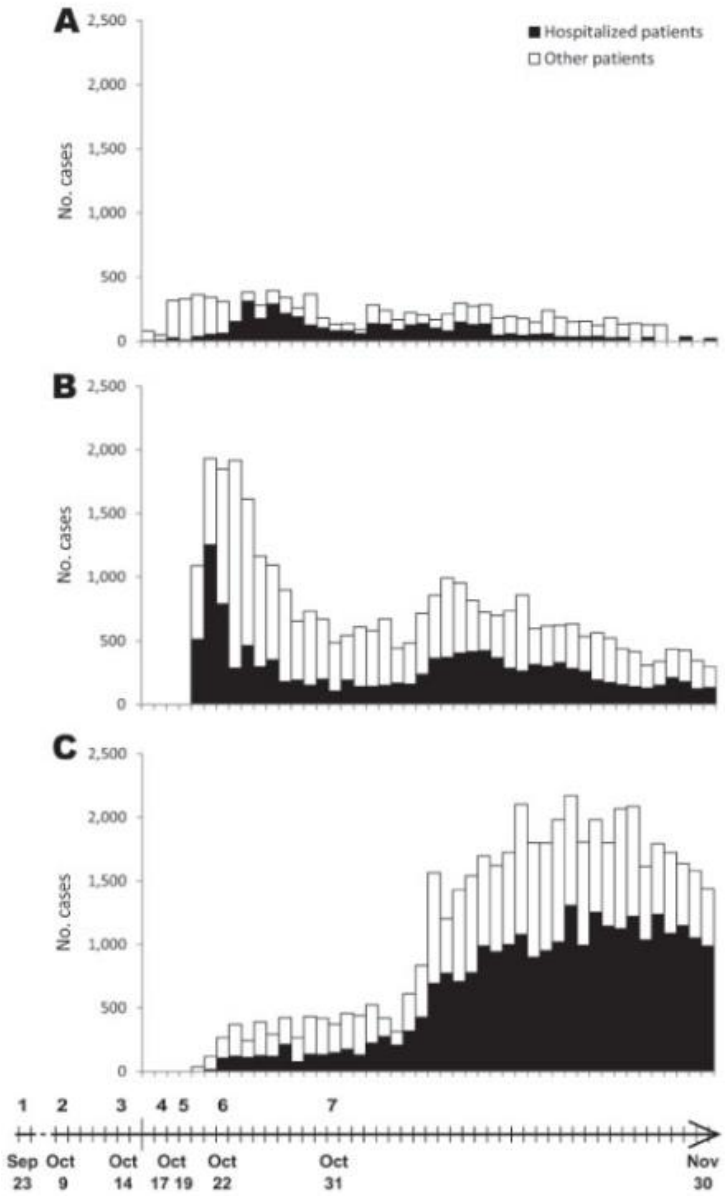


Figure 2. Cholera cases by date of onset of the epidemics and major related events, Haiti. A) Cases in Mirebalais, commune hosting the first cases of cholera; B) cases in seven communes simultaneously struck on October 20 (St-Marc, Dessalines, Desdunes, Grande Saline, Lestere, Petite-Riviere-de-l'Artibonite, Verrettes); C) cases in other communes. Timeline at bottom indicates 1) cholera outbreak in Kathmandu, Nepal; 2) first arrival of newly incoming Nepalese soldiers in Meille; 3) first cases in Meille; 4) first death registered in Mirebalais hospital (patient from Meille); 5) initiation of epidemic investigations and spread into the Artibonite delta; 6) epidemiologic confirmation of cholera cases in Meille; 7) United Nations camp sanitary dysfunction no longer observed.

IMMUNOLOGIST

Pathogens in focus, *Vibrio cholerae*: Cholera toxin
Author: Vanden Broeck et al. (2007)
DOI: 10.1016/j.biocel.2007.07.005

Overview of Pathogenesis
This post-translational modification is critical for full activity of the toxin, leading to an increase in cAMP-production. This causes massive secretion of electrolytes and water into the intestinal lumen, paralleled by excretion of the bacteria. The patient's stool bears resemblance to rice water and can amount to over 10 L a day (Sanchez & Holmgren, 2005). Feaco-oral transmission of *V. cholerae* is the main transmission channel of the pathogen, often resulting in violent outbursts.

Treatment of cholera relies on forced rehydration of the patient, orally or intravenously, often in combination with electrolytes and antibiotics (Guerrant, Carneiro- Filho, & Dillingham, 2003). From a prophylactic point of view, administration of killed *V. cholerae* bacteria or attenuated strains could serve as a vaccine.

A major breakthrough was the observation that more powerful immune responses could be elicited using combinations of killed whole cell *V. cholerae* preparations with an excess of purified or recombinant CT-B sub-unit. Currently, the most efficient vaccine is based on these findings and protects adults up to 2 years after immunization. (Hill, Ford, & Lalloo, 2006).

Clinical Applications
CT generates extremely potent anti-toxin anti-bodies following systemic immunization, even in the absence of classical adjuvants, potentially providing a strategy to enhance immunogenicity of orally delivered antigens. The obvious problem of using CT as an adjuvant is the potential for inducing pathological diarrhoea. However, CT acts as an adjuvant at doses lower than that required for the induction of toxic side effects, and importantly the B subunit has been identified as the immune adjuvant agent, at least when administered intranasally or parenterally. In orally delivered vaccines, CT-B alone displayed no immunological capacities, which might be explained by lack of stability during passage of the stomach, which requires the presence of the A-subunit (Hirst et al., 2002).

Coupling of the antigen to CT-B, prior to oral administration, can induce immunological tolerance. In this respect, promising results have been obtained in the area of autoimmune disorders, where coupling of an auto-antigen to CT-B induced tolerance e.g. the oral use of an insulin/CT-B complex as an effective therapy against diabetes in mice. Although no precise mechanism has been put for- ward to explain these findings, the need to directly couple antigen and CT-B stresses its role as a carrier, shuttling antigen into tolerance-inducing pathways via GM1 -receptor-mediated uptake across the intestinal epithelium. Moreover, the observation that CT-B, even in the absence of the auto-antigen, can induce tolerance suggests that CT-B has distinct immunological capacities way beyond that of a carrier function.

A Therapeutic Chemical Chaperone Inhibits Cholera Intoxication and Unfolding/Translocation of the Cholera Toxin A1 Subunit

Author: Taylor et al. (2011)*

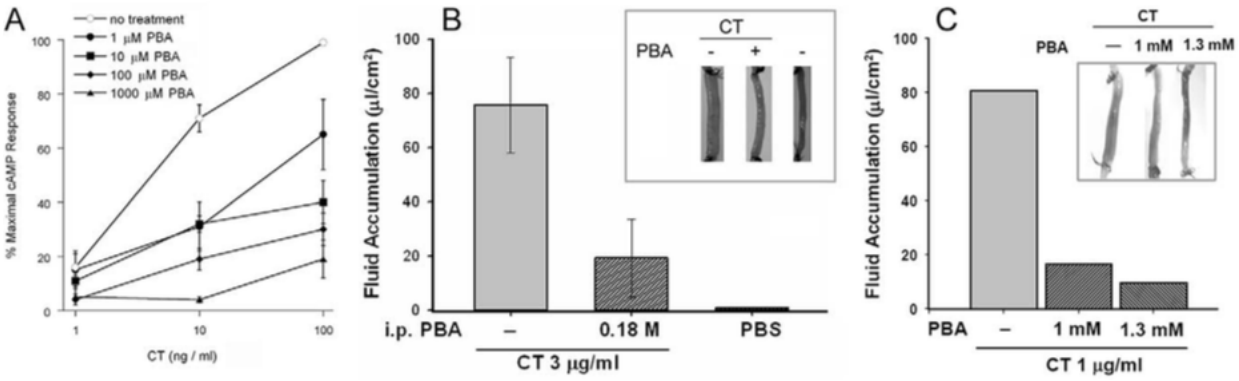
DOI: 10.1371/journal.pone.0018825

*Research carried out at UCF.

Introduction

The overall aim of this work was to determine if a therapeutic chemical chaperone could be used to block the cytopathic effects of CT. Here, we report that 4-phenylbutyric acid (PBA) inhibits the thermal unfolding of CTA1, the ER-to-cytosol translocation of CTA1, and CT intoxication. PBA is a chemical chaperone and a therapeutic agent approved by the Food and Drug Administration (FDA) for the management of urea cycle disorders [17,18]. The therapeutic value of PBA in treating these disorders relates to its function as an ammonia scavenger rather than its ability to function as a chemical chaperone. In vitro, PBA bound to the CT holotoxin and the CTA1 polypeptide with nM affinity but did not bind to the CTB pentamer. In vivo, PBA effectively blocked fluid accumulation in the physiological ileal loop model of CT intoxication. PBA could thus represent a novel therapeutic agent for the prevention or treatment of cholera.

Results



The PBA-induced inhibition of toxin translocation would prevent CT-A1 from entering the cytosol where its $G_{s\alpha}$ target is located. PBA should therefore inhibit the cytopathic effects of CT … (A) PBA- treated cells were highly resistant to CT. At the EC_{50}, cells exposed to just 1 mM PBA were 10-fold more resistant to CT than the untreated control cells. Intoxicated cells treated with 10 mM PBA did not reach the half-maximal cAMP value of the control cells. Even at the highest toxin concentration of 100 ng/ml, cells treated with 10 mM PBA only produced 40% of the maximal cAMP signal obtained from the control cells. Cells treated with 10 mM PBA therefore required at least 25-fold higher concentrations of toxin to reach the EC_{50} obtained for the untreated control cells. Dose-dependent disruptions to CT intoxication were also recorded for cells exposed to 100 or 1000 mM PBA. (B) To examine the therapeutic potential of PBA as an anti-CT agent, we employed a physiological ileal loop model of CT intoxication. Rats were injected intraperitoneally with a concentration of PBA. As shown in Figure 8B, the intestinal distension and fluid accumulation induced by CT was dramatically attenuated in PBA-treated rats. The effects of intoxication were also substantially reduced when PBA and CT were co-injected directly into the intestinal loops (Fig. 8C). For both methods of drug delivery, CT-

induced fluid accumulation was reduced by about 75% in comparison to control rats that were exposed to CT but not PBA. These results demonstrated that PBA, an FDA-approved therapeutic, can provide substantial in vivo protection against CT.

Haitian cholera outbreak—–United Nations admits involvement

Author: Qadir et al. (2016)
DOI: 10.1016/j.jiph.2016.10.003

The United Nation's recent admittance of its involvement in the Haiti cholera outbreak has led to harsh criticism of the world body. Despite claiming immunity against legal action, it took more than five years for the UN to accept that it played a key role in bringing cholera to the region [1]. The first case of cholera was seen in Haiti in October 2010, following a devastating earthquake that struck earlier in the year [2]. By December 2010, 121,518 cases had been reported with the death toll rising to 2591. Not only had the epidemic affected all 10 provinces of Haiti, but had also by then spread to the adjoining Dominican Republic and Florida, United States [3].
Five years later, almost 10,000 people have died, and hundreds of thousands have been affected by the disease, with 14,000 cases reported in 2016 alone [2]. When investigated, two possible hypotheses arose, regarding how the bacteria, and consequently the potentially life-threatening diarrheal disease reached Haiti.

The initial 'environmental' hypothesis claimed that the virulent strain responsible for the epidemic had previously existed in the waters around Haiti, and the January 2010 earthquake had caused the bacteria to contaminate the drinking water. DNA sequencing of stool samples from Haitian patients supported this; studies revealed the presence of both infectious and noninfectious environmental strains, but did not ascertain which of the two caused the disease [2].

The second hypothesis, the 'peacekeeper's hypothesis', provided a more believable explanation. The earthquake had attracted international aid from many organizations, including the United Nations, which sent troops to the region. The last troop of peacekeepers had come from Nepal, a cholera endemic region. They reached Haiti in October, just days before the first case was reported.

On testing for similarities in DNA sequence between five Nepalese strains, and three Haitian strains, it was discovered that the strains differed only at 1—2 base pairs; practically confirming that the strains found in Haiti had come from Nepal[4]. Another supportive study also established similarities in antimicrobial resistance activity in Haitian and Nepalese strains[5].

The scientific community and the world, have long since concluded that the peacekeeper's hypothesis is the more plausible explanation. Had the UN screened its Nepalese peacekeepers, for a cost of only about $2000, this crisis could have been averted[1]. Since accepting responsibility, the UN has promised to raise funds and use them to implement large-scale vaccination, and treatment of Haitian people, but has failed to make much progress in doing so.

CHOLERA

Cholera transmission: the host, pathogen and bacteriophage dynamic

Author: Nelson et al. (2009)
DOI: 10.1038/nrmicro2204

Table 1 | **Clinical spectrum of *Vibrio cholerae* infection**

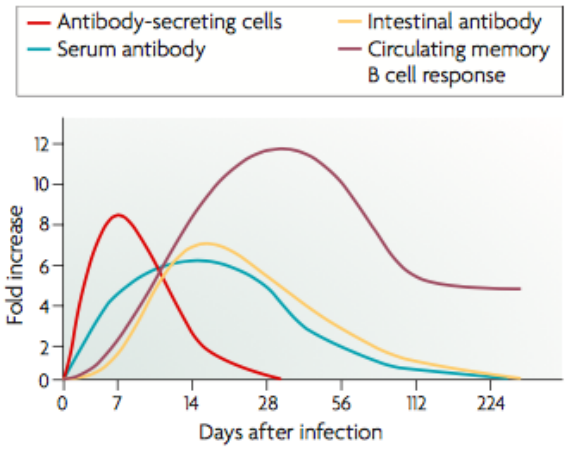
	Asymptomatic infection	Mild infection	Severe infection
Symptoms	None	Diarrhoea*	Vomiting and profuse diarrhoea
Dehydration	None	None to mild	Moderate to severe (hypovolaemic shock)
Stool characteristics	Normal	Loose or watery	Rice water
Vibrios per gram of stool	Up to 10^5	Up to 10^8	10^7 to 10^9 in stool (and vomitus)
Treatment	None	Oral rehydration solution (ORS)	ORS, intravenous fluids and antibiotics‡
Mortality	None	None	Untreated: up to 50% Treated: less than 1%

*Mild symptoms of *Vibrio cholerae* infection are indistinguishable from those of numerous other infectious causes of gastroenteritis.
‡Antibiotics shorten the duration of symptoms and lessen the total fluid requirement but are not strictly necessary.

Box 1 | **The human immune response to *Vibrio cholerae***

Innate response

Cholera is thought to be a prototypical non-inflammatory infection. There are often no gross changes to the intestinal mucosa or the architectural integrity of the small bowel. However, there is upregulation of pro-inflammatory cytokines (including interleukin-1β and tumour necrosis factor) the expression of diverse bactericidal proteins and migration of neutrophils to the lamina propria during acute cholera. Natural variability in the innate immune response may affect susceptibility, suggested by the finding that a polymorphism in the promoter region of the *LPLUNC1* (long palate, lung and nasal epithelium carcinoma-associated protein 1) gene is associated with increased risk of cholera.



Adaptive response

How the adaptive immune response to cholera mediates protection against subsequent disease is unknown. As *Vibrio cholerae* is non-invasive, it has been suggested that intestinal secretory immunoglobulin A (sIgA) protects against colonization of the mucosa. Approximately 8 days after the onset of cholera, there is a peak in circulating *V. cholerae* antigen-specific lymphocytes that express gut-homing chemokine-receptors (see the figure)[112]. These lymphocytes soon become undetectable in the blood, as they return to the intestinal mucosa, where they lead to a rise in intestinal sIgA secretion. Serum antibody responses such as the vibriocidal antibody response also peak 1–3 weeks after infection. Although high serum titres of vibriocidal antibody and cholera toxin-specific IgA are correlated with protection against infection, these antibodies decrease to baseline levels by a year after infection, long before protective immunity to cholera wanes. Similarly, in *V. cholerae*-infected volunteers, mucosal sIgA levels decrease to baseline levels in months. However, when volunteers who no longer have detectable antibodies are re-challenged with *V. cholerae* antigens, they demonstrate a persistent ability to mount an anamnestic immune response, developing peak intestinal antibody secretion as rapidly as within three days. Therefore it is possible that the rapidity of the anamnestic response on re-exposure, rather than preformed antibodies, may mediate protection against cholera. This is supported by recent evidence that cholera induces a memory B cell response that is detectable for at least 1 year after cholera infection[113].

HISTORICAL CONTEXT

Zika Virus 6 Months Later

Author: Frieden et al. (2016)
DOI: 10.1001/jama.2016.11941

On January 15, 2016, the Centers for Disease Control and Prevention advised pregnant women not to travel to areas where the Zika virus was spreading. Six months later, more than 60 countries or territories have reported new local transmission of Zika. By August 4, 2016, nearly 1700 cases of travel-associated Zika infection, including 479 in pregnant women, had been reported in the continental United States; Puerto Rico is experiencing rapid and extensive spread of the epidemic.[1]

The association between Zika infection (both symptomatic and asymptomatic) and serious birth defects, including microcephaly, has been confirmed.[2] Sexual transmission of Zika from both male and female partners can occur, and the virus may be able to remain viable in semen for months. The competent vectors - *Aedes aegypti* as well as the less efficient vector *Aedes albopictus* - put 30 and 41 US states, respectively, at risk for local mosquito-borne transmission of Zika. Risk of microcephaly after Zika infection early in pregnancy may range from 1% to 13%; the full spectrum of congenital Zika virus syndrome is not known, nor is it known whether infants exposed to Zika during pregnancy who appear healthy at birth will have neurologic or other problems.

For the public health community, Zika represents an unprecedented emergency. Never before, to our knowledge, has a mosquito-borne virus been associated with human birth defects or been capable of sexual transmission. The effects of brain damage due to microcephaly and consequences of other Zika-related birth defects are likely devastating, lifelong, and costly. However, most people infected with Zika have no symptoms, and concern among the general public has been muted even in some affected locales. Zika is a silent epidemic.

Puerto Rico's Ongoing Battle Against Zika: A Plea for Washington's Attention

Author: Lewis & Shaw (2017)

Puerto Rico is in the midst of a large epidemic of Zika. Experience with chikungunya, another newly introduced virus spread by *A. aegypti*, as well as blood screening, case reports, and testing of pregnant women, suggest that 25% of Puerto Rican individuals, including an estimated 6000 to 11 000 pregnant women, may be infected by Zika this year.

While it is true that the Zika epidemic has reached other parts of the hemisphere (a March 2017 incidence report covering December 2015 to the present cited 993 cases in the US Virgin Islands, for example), Washington has been unable to devise adequate preventative measures and properly protect the people of Puerto Rico, making the territory a hotspot for contracting the disease. On March 15, 2017, the Center for Disease Control (CDC) released a report identifying over 37,000 cases of the virus present in Puerto Rico since it first emerged in 2015. While these numbers are staggering, more alarming still is the fact that the disease affects the most vulnerable

of Puerto Rico's citizens: pregnant mothers and their infants. In July of 2016, the CDC disclosed a study revealing that at least 670 pregnant women tested positive for the disease between November of 2015 and July of 2016. While it could be argued that this number reflects only a slim percentage of the cases identified, the release also suggested that the total number was likely far higher, but due to Zika's asymptomatic nature, it would be nearly impossible to confirm.

As a testimony to the lackadaisical nature of Washington's response, in 2016, for eight months, President Obama repeatedly urged Congress to pass the "Stopgap Spending" Bill to devote federal funds to Zika prevention and research with no success. At the same time, Zika cases simultaneously rose exponentially in Puerto Rico and the southern United States. The fight for funding finally paid off in the form of a $1 billion USD package in September of 2016, aimed at combating the disease, but given the lapsed time without action, thousands were put at risk as a result of lack of timely legislative action.

Currently, Puerto Rico is in the middle of a major economic crisis. The US territory has roughly $70 billion in debt, an unemployment rate more than twice the US average and a hugely underfunded Medicaid insurance program for those who cannot afford basic health care. Some argue these conditions were brought on by legislation that unfairly and unequally treats U.S. territories compared to states, citing such instances as the U.S. Congress's early 2000's cuts to Puerto Rican business taxes, forcing Puerto Rico to continually borrow money to make up for the lack of revenue. … In a 2015 article from the *American Journal of Medicine* (AJM), it was reported that the U.S. government cut private healthcare funding in Puerto Rico by 11 percent. As a result, a *Reuters* investigative report from 2016 estimated that "working physicians now number 9,000, down 36 percent from a decade ago, a percentage decline four times greater than that for Puerto Rico's overall population."

Puerto Rico Braces for Its Own Zika Epidemic
Author: McNeil (2016)

On an inexorable march across the hemisphere, the Zika virus has begun spreading through Puerto Rico, now the United States' front line in a looming epidemic. The outbreak is expected to be worse here than anywhere else in the country. The island, a warm, wet paradise veined with gritty poverty, is the ideal environment for the mosquitoes carrying the virus. The landscape is littered with abandoned houses and discarded tires that are perfect breeding grounds for the insects. Some homes and schools lack window screens and air-conditioning, exposing residents to almost constant bites.

The economy is in shambles, and thousands of civic workers needed to fight mosquitoes have been laid off. The chemical most often used against the adult pests no longer works, and the one needed to control their larvae has been pulled from the market by regulators.

A quarter of the island's 3.5 million people will probably get the Zika virus within a year, according to the Centers for Disease Control and Prevention, and eventually 80 percent or more may be infected. "I'm very concerned," Dr. Thomas R. Frieden, the C.D.C. director, said in an

interview after a recent three-day visit to Puerto Rico. "There could be thousands of infections of pregnant women this year."

The epidemic is unfolding in one of the country's most popular vacation destinations, where planes and cruise ships disembark thousands of tourists daily. Anyone could carry the virus back home, seeding a mosquito-borne outbreak or transmitting it sexually. Health officials here have begun intensive efforts to stop the virus, which has been linked to abnormally small heads and brain damage in babies born to infected mothers, and to paralysis in adults.

Trucks are rumbling through communities, shrouding them in insecticide. Schools are being outfitted with screens to protect children, and hundreds of thousands of old tires have been retrieved and disposed of. Officials have warned scores of island towns that they must clean up the detritus in which standing water collects, incubating new mosquitoes. And the C.D.C. is preparing to spend tens of millions of dollars to blunt the spread of the virus. Still, officials are not optimistic that they will succeed.

Zika Virus
Author: Petersen et al. (2016)
DOI: 10.1056/NEJMra1602113

In 1947, a study of yellow fever yielded the first isolation of a new virus, from the blood of a sentinel rhesus macaque that had been placed in the Zika Forest of Uganda. Zika virus remained in relative obscurity for nearly 70 years; then, within the span of just 1 year, Zika virus was introduced into Brazil from the Pacific Islands and spread rapidly throughout the Americas.[2] It became the first major infectious disease linked to human birth defects to be discovered in more than half a century and created such global alarm that the World Health Organization (WHO) would declare a Public Health Emergency of International Concern.[3]

MOLECULAR BIOLOGIST

Zika Virus

Author: Petersen et al. (2016)
DOI: 10.1056/NEJMra1602113

The mainstays of the routine diagnosis of Zika virus infection are the detection of viral nucleic acid by RT-PCR and the detection of IgM antibodies by IgM-capture enzyme-linked immunosorbent assay (MAC-ELISA). The detection of viral nucleic acid in serum provides a definitive diagnosis; however, in most instances viremia is transient, and diagnosis by RT-PCR has been most successful within 1 week after the onset of clinical illness.[67,101] In contrast, viral RNA was detected in serum approximately 10 weeks after infection in a pregnant woman whose fetus had evidence of congenital infection.[95] In addition, viremia is generally low level, which makes viral isolation from clinical samples difficult.[101] Although the precise timing of the onset and the duration of the IgM antibody response to Zika virus that is detectable by MAC-ELISA have not yet been defined, extensive experience with other, related flaviviruses suggests that IgM will appear as viremia wanes within the first week after symptom onset and will persist for several months.[102] Thus, RT-PCR testing of serum samples obtained within the first week of clinical illness and MAC-ELISA testing of samples that are not tested by RT-PCR or that are found to be negative by RT-PCR are likely to have the highest diagnostic yield.[103]

The considerable cross-reactivity of flavivirus antibodies presents major challenges for the interpretation of serologic test results. For example, recent Zika virus infection may also evoke a positive MAC-ELISA result for dengue.

N-Methyl-D-Aspartate (NMDA) Receptor Blockade Prevents Neuronal Death Induced by Zika Virus Infection

Author: Costa et al. (2017)
DOI: 10.1128/mBio.00350-17.

Recent studies have shown that ZIKV has extensive tropism to the central nervous system (CNS) and causes significant neurodegeneration, especially of neural progenitor cells [19–22]. These neurodegenerative effects appear to account for the neurological disorders associated with ZIKV infection [7, 12, 23].

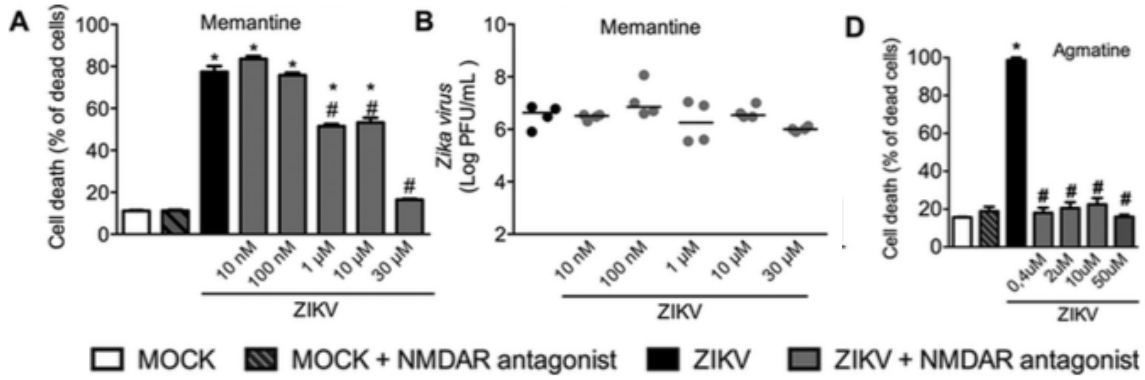
Glutamate is the main excitatory neurotransmitter in the brain and plays a pivotal role during neurodegenerative processes [24–26]. There are two types of glutamate receptors: ionotropic and metabotropic [27]. Several studies indicate that glutamatergic overstimulation via activation of ionotropic glutamate receptors leads to excitotoxicity, which promotes neuronal calcium overload and, consequently, neurodegeneration [28]. Here, we hypothesize that *N*-methyl-D-aspartate receptor (NMDAR) blockade by memantine can avoid the death of nearby neurons and decrease neurodegeneration and neuroinflammation associated with ZIKV infection.

ZIKV induces massive neuronal damage of primary cultured neurons. Mechanisms underlying cell death induced by ZIKV have not been fully elucidated. … We evaluated the

ability of ZIKV to infect and replicate in these primary cultures. Of note, virus replication in neurons was about 2- to 3-fold higher than that in glial cells, suggesting increased susceptibility of neuronal cells to ZIKV.

NMDAR blockade prevents neuronal death induced by ZIKV. Neurodegeneration is a hallmark of neurodegenerative diseases such as Alzheimer's disease (AD) [38–40]. In these neurodegenerative conditions, neurological alterations appear to be closely associated with NMDAR-dependent neuronal death.

NMDAR blockade prevents neuronal death induced by ZIKV. Our results indicate that memantine treatment was able to prevent neuronal cell death induced by ZIKV (**Figure 5A**), especially at the highest tested dose (30 M). At these concentrations, memantine did not interfere with the ability of the virus to replicate in neuronal cells *in vitro* (**Figure 5B**). Agmatine emerged as a neuromodulator and a promising agent to manage various central nervous system disorders by modulating the nitric oxide (NO) pathway, glutamate NMDARs, and oxidative stress [42]. **Figure 5D** shows the effect of agmatine treatment on ZIKV- infected neurons. Interestingly, agmatine treatment prevented neuronal death in all tested doses (**Figure 5D**).



Zika Virus Vaccines — A Full Field and Looking for the Closers
Author: Thomas (2017)
DOI: 10.1056/NEJMcibr1701402

Two recent reports describing the successful testing of experimental ZIKV vaccines in animal models — one by Pardi et al.[1] and another by Richner et al.[2] — are welcome news. Both groups engineered messenger RNAs (mRNAs) with sequences encoding the ZIKV precursor membrane (prM) glycoprotein and envelope (E) glycoprotein. The E protein is critical to viral attachment, entry, and replication in the infected host (**Figure 1A**), which makes it a rational vaccine target. Neutralizing antibodies directed against the E protein have been identified as correlates of protection for vaccines directed against other flaviviruses, such as the Japanese encephalitis, yellow fever, and tickborne encephalitis viruses.[3]

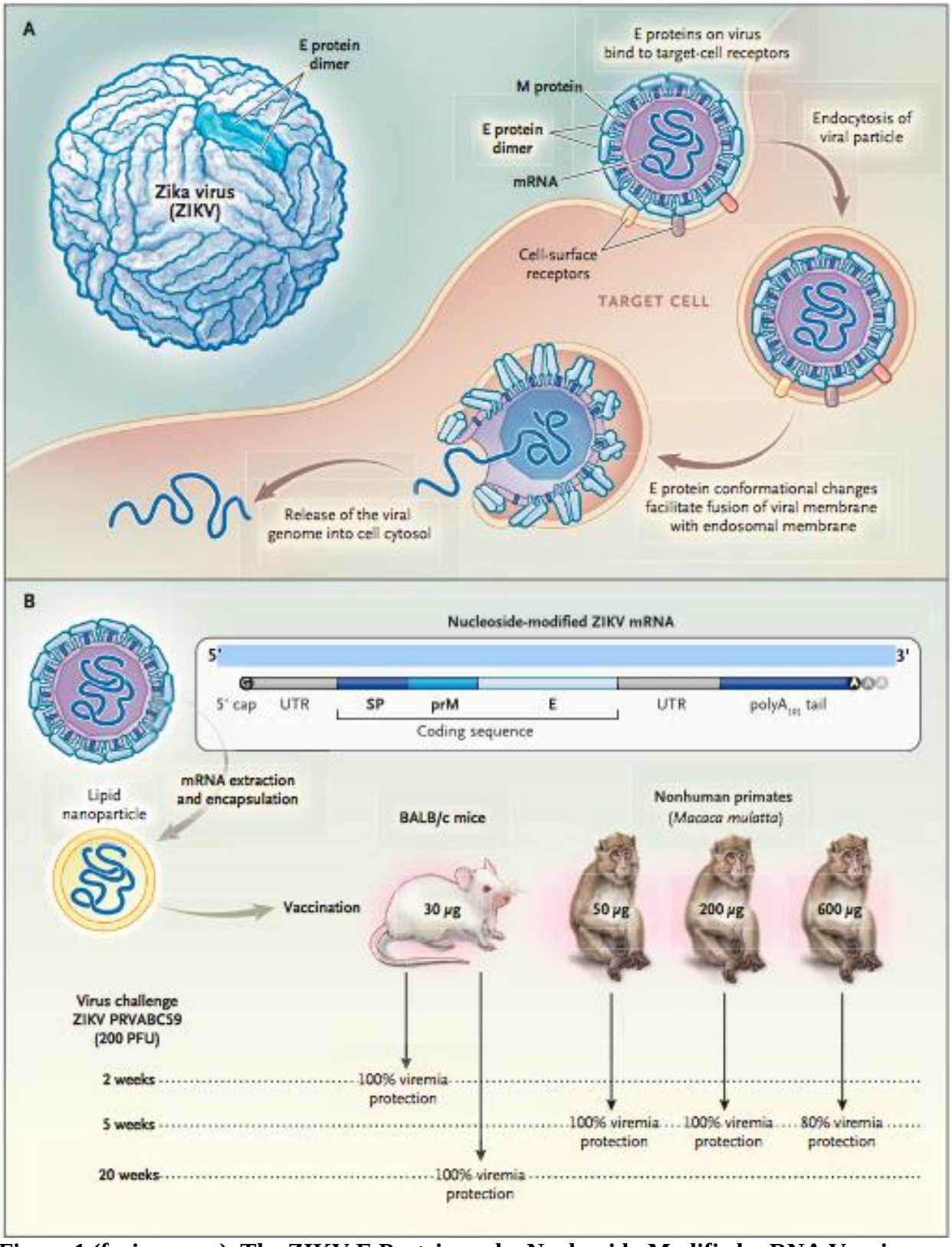


Figure 1 (facing page). The ZIKV E Protein and a Nucleoside-Modified mRNA Vaccine Candidate. Envelope (E) glycoprotein of the Zika virus (ZIKV) interacts with receptors on the surface of target cells (**Panel A**), promoting viral entry, processing, and ultimately replication.

The presence of a sufficient quantity of high-quality antibody directed against the E protein may neutralize the virus and reduce or prevent the replication process. Virus neutralization and decreased replication may abort infection or prevent or substantially attenuate disease. A vaccine capable of inducing robust neutralizing antibodies may also reduce the likelihood of transmission between persons and populations. Pardi and colleagues[1] based their vaccine candidate on messenger RNA (mRNA) encoding for French Polynesian 2013 ZIKV precursor membrane (prM) and E glycoproteins (**Panel B**). Nucleoside modification and the addition of lipid nanoparticles formed a ZIKV mRNA– lipid nanoparticle vaccine, which was tested in mice and in nonhuman primates. At varying points after vaccination, BALB/c mice and nonhuman primates were challenged with a 2015 Puerto Rican ZIKV strain. Neutralizing and binding antibodies developed after vaccination, and high levels of protection against challenge were found. PFU denotes plaque-forming units, SP signal peptide, and UTR untranslated region.

The Neurobiology of Zika Virus
Author: Li et al. (2016)
DOI: 10.1016/j.neuron.2016.11.031

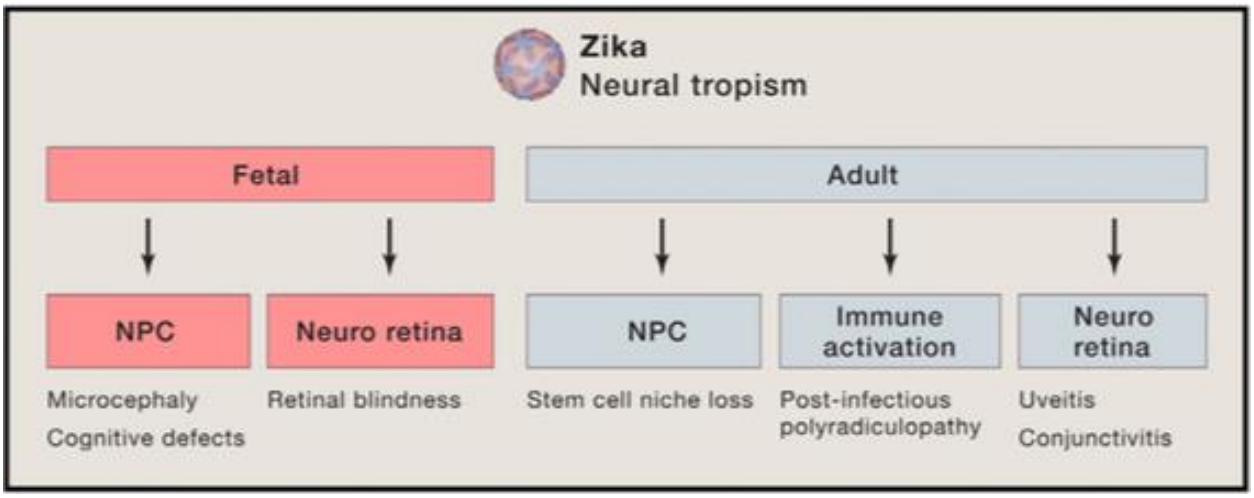


Figure 1. Neural Tropism for Zika Virus Determines Site of Pathology. ZIKV can cause neurological disease through direct infection of cells or through immune-mediated effects in the case of post-infectious polyradiculopathy (Guillain-Barre´ syndrome). In the fetus, ZIKV can infect neural progenitor and neural retinal cells, leading to microcephaly and retinal disease. The long-term consequences on cognition are not documented. In adults, infection of neural progenitors in stem cell niches can lead to loss of these populations. Adults can also display post-infectious polyradiculopathy, presumably due to immune activation, as well as uveitis and conjunctivitis.

EVOLUTIONARY BIOLOGIST

Zika Virus

Author: Petersen et al. (2016)
DOI: 10.1056/NEJMra1602113

Zika virus is a flavivirus, in the family Flaviviridae. Although Zika virus was isolated on several occasions from *Aedes africanus* mosquitoes after its discovery in 1947,[4] there initially was no indication that the virus caused human disease. Nevertheless, a serosurvey involving residents of multiple areas of Uganda revealed a 6.1% seroprevalence of antibodies against Zika virus, which suggested that human infection was frequent.[5] Additional serosurveys indicated a much broader geo- graphic distribution of human infection, including Egypt,[6] East Africa,[7] Nigeria,[8] India,[9] Thailand,[10] Vietnam,[10] the Philippines,[11] and Malaysia (near Kuala Lumpur and in East Malaysia [Sabah and Federal Territory of Labuan]).[12]

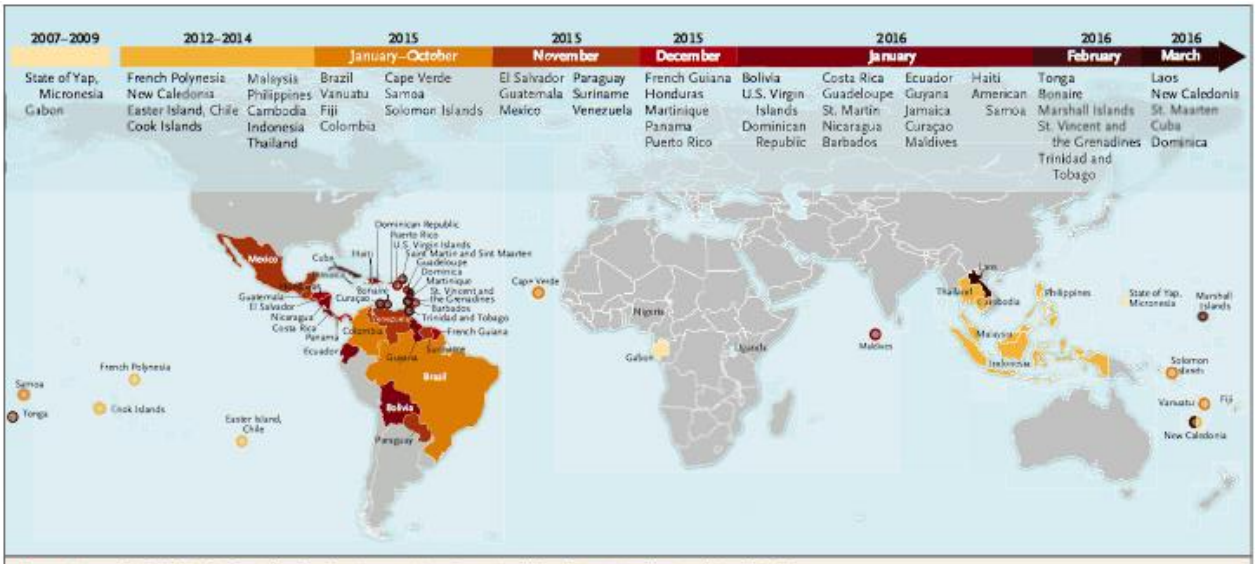


Figure 1. Areas in Which Zika Virus Infections in Humans Have Been Noted in the Past Decade (as of March 2016).
Only sporadic infections have occurred in Southeast Asia, the Philippines, and Indonesia.

Zika virus evolution and spread in the Americas

Author: Metsky et al. (2017)
DOI: 10.1038/nature22402

To investigate the spread of ZIKV in the Americas we performed a phylogenetic analysis of the 110 genomes from our dataset, together with 64 published genomes available on NCBI GenBank and in refs 10 and 11 (**Figure 2A**). Our reconstructed phylogeny (**Figure 2A**), which is based on a molecular clock (Extended Data Fig. 2), is consistent with the outbreak having originated in Brazil[12]: Brazil ZIKV genomes appear on all deep branches of the tree, and their most recent common ancestor is the root of the entire tree. We estimate the date of that common ancestor to have been in early 2014 (95% credible interval (CI) August 2013 to July 2014). The shape of the tree near the root remains uncertain (that is, the nodes have low posterior probabilities) because

there are too few mutations to clearly distinguish the branches. This pattern suggests rapid early spread of the outbreak, consistent with the introduction of a new virus to an immunologically naive population. ZIKV genomes from Colombia (n = 10), Honduras (n = 18), and Puerto Rico (n = 3) cluster is in distinct, well-supported clades. We also observed a clade consisting entirely of genomes from patients who contracted ZIKV in one of three Caribbean countries (the Dominican Republic, Jamaica, and Haiti) or the continental United States, containing 30 of 32 genomes from the Dominican Republic and 19 of 20 from the continental United States. We estimated the within-outbreak substitution rate to be 1.15×10^{-3} substitutions per site per year (95% CI (9.78×10^{-4}, 1.33×10^{-3})), similar to prior estimates for this outbreak12. This is 1.3–5 times higher than reported rates for other flaviviruses[13], but is measured over a short sampling period, and therefore may include a higher proportion of mildly deleterious mutations that have not yet been removed through purifying selection.

Determining when ZIKV arrived in specific regions helps to elucidate the spread of the outbreak and track rising incidence of possible complications of ZIKV infection. The majority of the ZIKV genomes from our study fall into four major clades from different geographic regions, for which we estimated a likely date for ZIKV arrival.

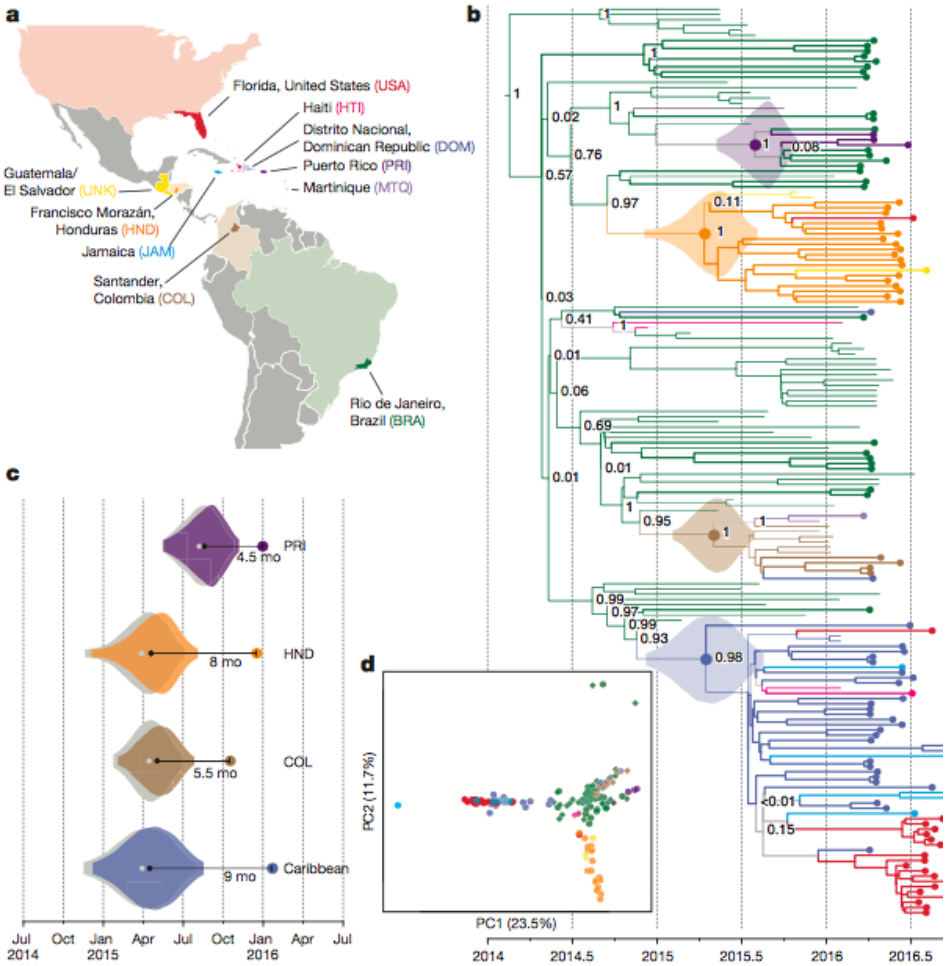
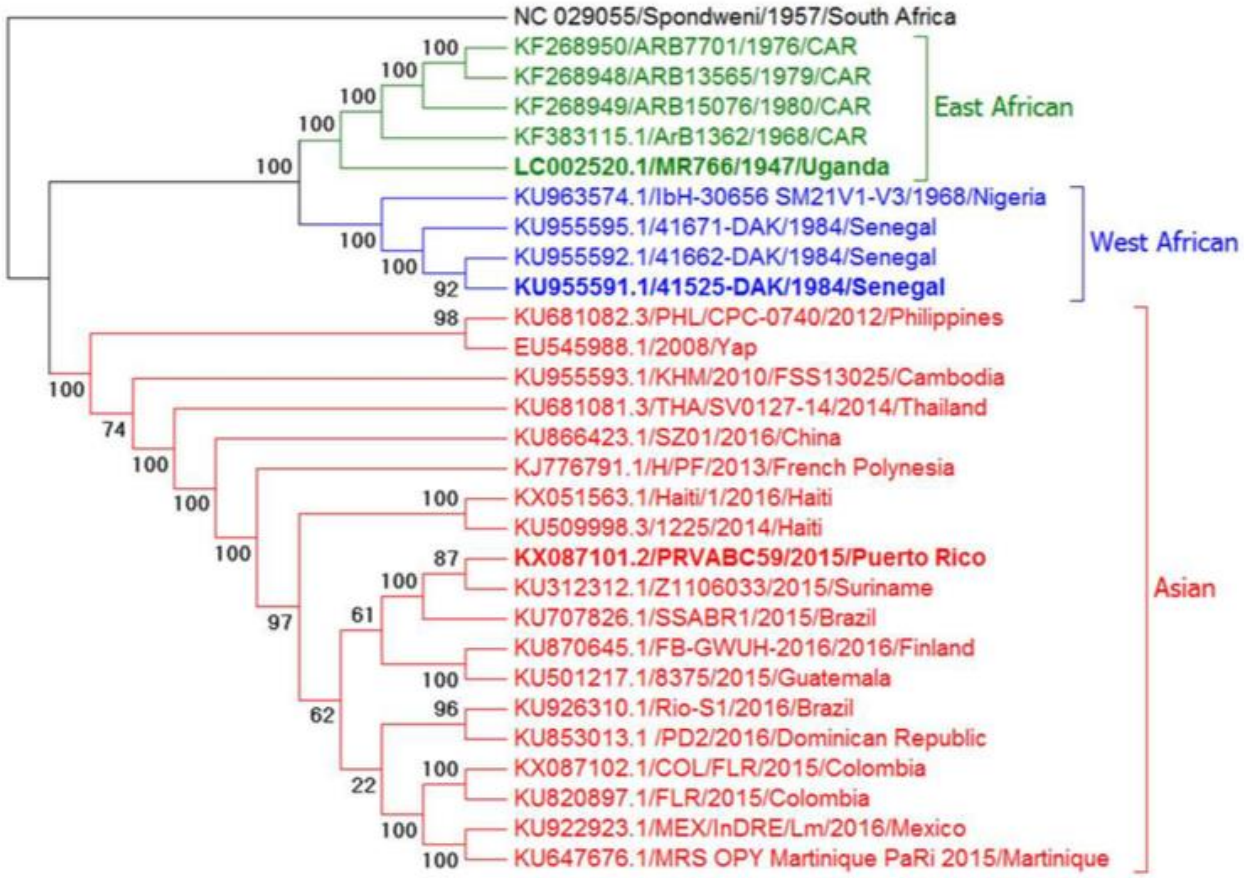


Figure 2 | Zika virus spread throughout the Americas. A. Samples were collected in each of the coloured countries or territories. Specific state, department, or province of origin for samples in this study is highlighted if known. **B.** Maximum clade credibility tree. Dotted tips, genomes

generated in this study. Node labels are posterior probabilities indicating support for the node. Violin plots denote probability distributions for the tMRCA of four highlighted clades. **C.** Time elapsed between estimated tMRCA and date of first confirmed, locally transmitted case. Colour, distributions based on relaxed clock model (also shown in **B**); grey, strict clock. Caribbean clade includes the continental United States. **D.** Principal component analysis of variants. Circles, data generated in this study; diamonds, other publicly available genomes from this outbreak. Percentage of variance explained by each component is indicated on axis.

Vector Competence of American Mosquitoes for Three Strains of Zika Virus
Author: Weger-Lucarelli et al. (2016)
DOI: 10.1371/journal.pntd.0005101



As previously reported [15], ZIKV sequences separate into three distinct genetic clades; West African, East African, and Asian. For this study, we selected one strain from each clade: strain 41525 from Senegal represented the West African clade, prototype strain MR766 from Uganda represented the East African clade, and the currently-circulating strain from the Americas, PRVABC59 from Puerto Rico, belongs to the Asian clade. The three viruses will be herein referred to as PRVABC59 (Americas), 41525 (West African), and MR766 (East African).

African strains used in this study out-competed the American strain *in vitro* in both mammalian and mosquito cell culture. West and East African strains of ZIKV tested here were more

efficiently transmitted by *A. aegypti* from Mexico than was the currently circulating American strain of the Asian lineage. Long-established laboratory colonies of *Culex* mosquitoes were not efficient ZIKV vectors. These data demonstrate the capacity for additional ZIKV strains to infect and replicate in American *Aedes* mosquitoes and suggest that neither enhanced virus replicative fitness nor virus adaptation to local vector mosquitoes seems likely to explain the extent and intensity of ZIKV transmission in the Americas.

STATISTICIAN

Estimating the Number of Pregnant Women Infected With Zika Virus and Expected Infants With Microcephaly Following the Zika Virus Outbreak in Puerto Rico, 2016

Author: Ellington et al. (2016)
DOI: 10.1001/jamapediatrics.2016.2974

Pregnant Population

The expected number of women who become pregnant each month was estimated using a binomial process with a rate of one-twelfth of the 2015 live birth rate in Puerto Rico (written communication with PRDH; May 2016) and the 2015 US Census estimate for the population of Puerto Rico (Table 1).[39]

Risk for Microcephaly

Congenital microcephaly was defined as clinically diagnosed microcephaly and did not include microcephaly based solely on head circumference. In 2013-2015, the annual rate of congenital microcephaly ranged from 2.6 to 5.5 per 10 000 live births in Puerto Rico (written communication with PRDH Birth Defects Surveillance Program; May 2016). Therefore, we used a uniform uncertainty distribution of 2 to 6 microcephaly cases per 10 000 live births. The risk for microcephaly given maternal ZIKV infection during pregnancy was based primarily on estimates derived from Bahia, Brazil.[18] These data indicated a strong association between maternal ZIKV infection in the first trimester and subsequent risk for microcephaly. We used a 1% to 13% risk for microcephaly given first trimester maternal ZIKV infection with a uniform distribution to reflect uncertainty.[18] Available data are limited but indicate that the risk for microcephaly associated with infection in the second and third trimesters of pregnancy is lower or might be no greater than the baseline microcephaly risk. Therefore, we set the most likely risk for microcephaly in these trimesters to 0.03% (range, 0%-0.7%) and 0% (range, 0%-0.2%), respectively.

Following the ZIKV Outbreak in Puerto Rico, 2016

Outcome	Median (IQR)
Women with ZIKV infection during pregnancy[a]	7800 (5900-10 300)
Cases of microcephaly due to ZIKV infection during pregnancy	180 (100-270)
Annual cases of microcephaly expected in the absence of ZIKV	12 (9-16)
Total expected cases of microcephaly[b]	190 (110-290)

Abbreviations: IQR, interquartile range; ZIKV, Zika virus.

[a] Estimate includes women infected with ZIKV in any trimester of pregnancy.

[b] Includes cases of congenital microcephaly expected in pregnant women with ZIKV infection and cases of congenital microcephaly expected in the absence of a ZIKV outbreak. The median and IQR reflect the samples from each distribution and thus do not reflect the exact sum of the independent median and IQRs.

Zika Virus

Author: Petersen et al. (2016)
DOI: 10.1056/NEJMra1602113

A. aegypti is thought to have high vectorial capacity (i.e., the overall ability of a vector species to transmit a pathogen in a given location and at a specific time) because it feeds primarily on humans, often bites multiple humans in a single blood meal, has an almost imperceptible bite, and lives in close association with human habitation.[48]

Both *A. aegypti* and *A. albopictus* bite primarily during the daytime and are widely distributed throughout the tropical and subtropical world. *A. albopictus* can exist in more temperate areas than *A. aegypti*, thus extending the potential range where outbreaks may occur. In the United States, *A. aegypti* is endemic throughout Puerto Rico and the U.S. Virgin Islands and in parts of the contiguous United States and Hawaii (**Figure 3**).[49] *A. albopictus* is widely distributed in the eastern United States and Hawaii. Nevertheless, in the contiguous United States, contemporary outbreaks of dengue, which has a transmission cycle similar to that of Zika virus, have occurred only in areas in which *A. aegypti* is endemic, which suggests that the potential for the transmission of Zika virus elsewhere is limited. In contrast, Hawaii has experienced contemporary dengue outbreaks in which *A. albopictus* was the vector.[50,51]

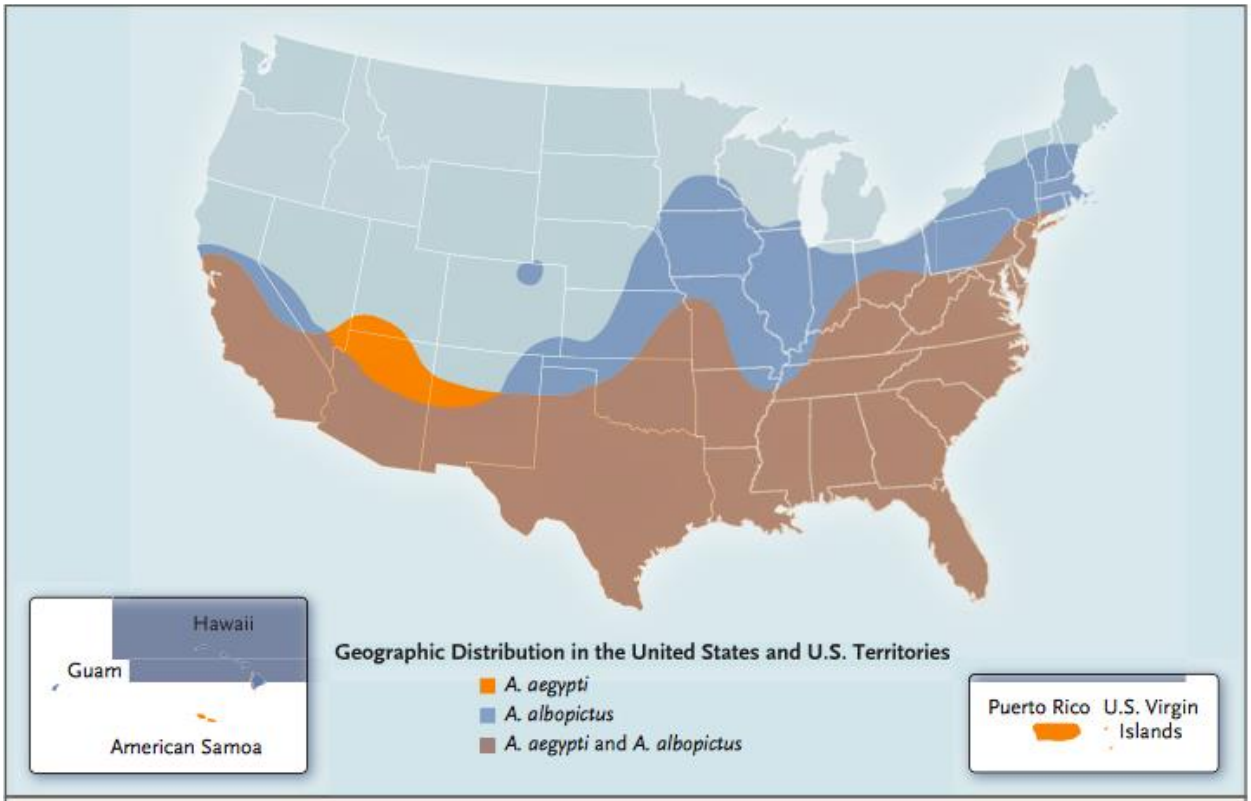
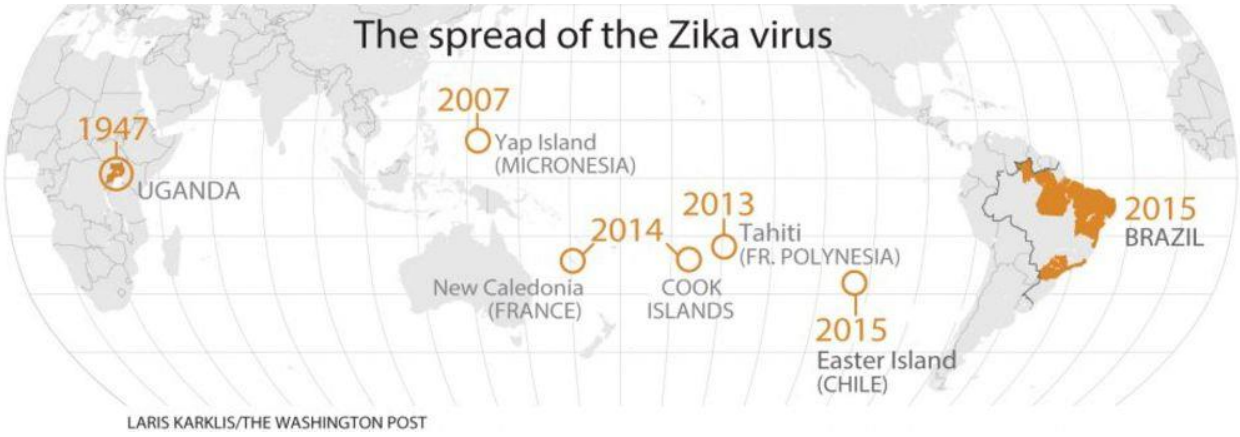


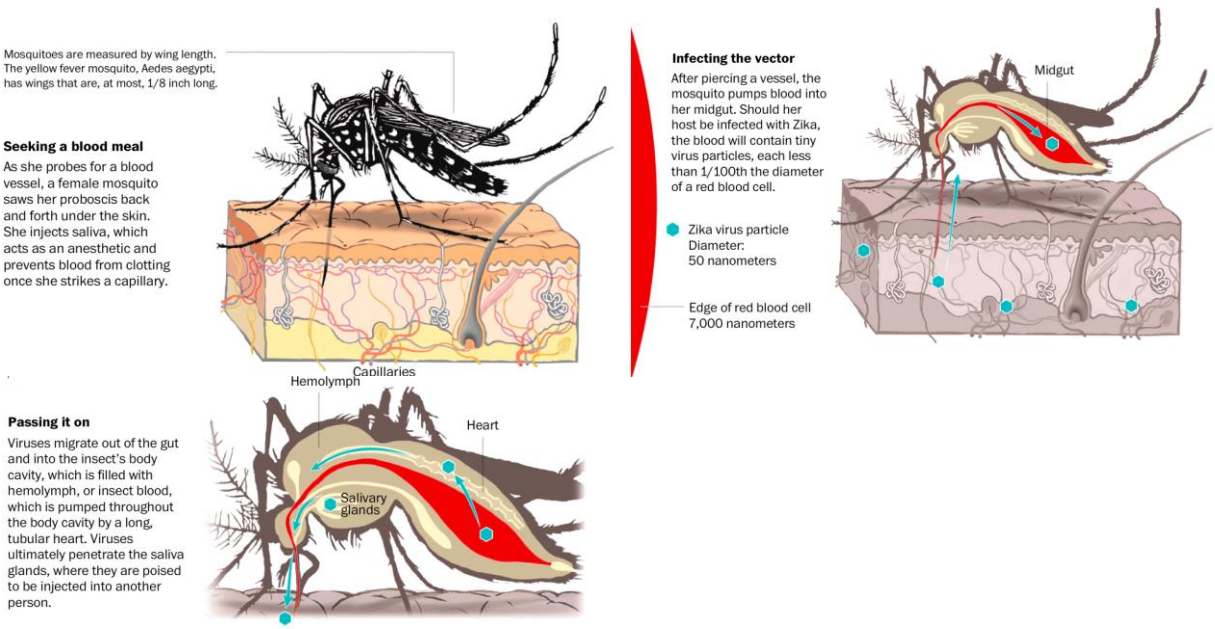
Figure 3. Approximate Ranges of *A. aegypti* and *A. albopictus* in the United States (as of March 2016).
These mosquitoes may not be present in all areas, and vector density may vary considerably within these ranges.

ZIKA

What you need to know about the Zika virus
Author: Berkowitz et al. (2016)



The *Aedes aegypti* mosquito is the perfect vector for spreading the virus. It picks up the virus from a human, then can infect another person as it injects saliva from its tiny syringe-like proboscis.



High infectious Zika virus loads have been detected in semen,[2] but data for viral persistence after symptomatic infections are scarce and even non-existent for asymptomatic ones, with the remaining key issue: how long does semen contain infectious Zika virus? As long as this question is unanswered, the adaptation of preventive measures such as the use of condoms and the abstinence of semen donation will be hampered.

Zika virus in semen and spermatozoa
Author: Mansuyt et al. (2016)
DOI: 10.1016/S1473-3099(16)30336-X

ZIKA

Here, we report the longitudinal follow up of Zika virus RNA in the semen of a 32-year-old man returning from French Guyana. At admission, the patient had moderate fever, maculopapular rash, myalgia, and arthralgia and was diagnosed for Zika virus infection upon detection of viral RNA in plasma and urine 2 days after onset of symptoms.

Semen (11 samples), blood (ten), and urine (five) were prospectively collected for 141 days after symptom onset. Zika virus RNA was detected on each semen sample and was still positive after 141 days, with viral load decreasing from 8·6 log copies per mL to 3·5 log copies per mL. It was detected until day 37 in both blood (2·5 log copies per mL) and urine (3·7 log copies per mL; figure)**.** Such a prolonged RNA Zika virus excretion is quite different from other flaviviruses, which are rapidly cleared by the immune response and for which the detection of viral nucleic acids is typically limited to a short window after symptom onset. In addition to the present case, we investigated five other symptomatic men for the presence of Zika virus RNA in semen; RNA was still detected 69 days and 115 days after the symptom onset in two patients, but not detected at day 20 in the other three individuals (data not shown). These data suggest that the length of Zika virus excretion varies, probably depending on viral and host characteristics, but long-lasting excretion might be frequent among adults who experienced a symptomatic infection**.**

IMMUNOLOGIST

Zika Virus

Author: Petersen et al. (2016)
DOI: 10.1056/NEJMra1602113

Acute Febrile Illness

The incubation period for Zika virus is unknown, but if it is similar to that of other mosquito-borne flaviviruses, it is expected to be generally less than 1 week. In one volunteer, a febrile illness of 4 days' duration developed 82 hours after subcutaneous inoculation of Zika virus.[67] Viremia was detected when symptoms were present, but not afterward. … Common symptoms were macular or papular rash (90% of patients), fever (65%), arthritis or arthralgia (65%), non-purulent conjunctivitis (55%), myalgia (48%), headache (45%), retro-orbital pain (39%), edema (19%), and vomiting (10%). … The rash is generally maculopapular and pruritic,[69] and fever, when present, is generally short-term and low-grade.[69]

Neurologic Complications

A temporal and geographic relationship has been observed between Guillain–Barré syndrome and Zika virus outbreaks in the Pacific and the Americas.[19,21,72-74] In the outbreak in French Polynesia, 38 cases of Guillain–Barré syndrome occurred among an estimated 28,000 persons who sought medical care.[19]

Adverse Fetal Outcomes

The full spectrum of fetal outcomes resulting from fetal Zika virus infection in humans is yet to be determined. Microcephaly is a clinical finding of a small head size for gestational age and sex and is indicative of an underlying problem with the growth of the brain.[87] The lack of consistent and standardized case definitions has challenged the accurate monitoring of microcephaly during the current Zika virus outbreak.[39] Centers for Disease Control and Prevention (CDC) guidance has recommended that microcephaly be defined as an occipitofrontal circumference below the third percentile for gestational age and sex.[88] The prevalence of microcephaly in the United States averages approximately 6 cases per 10,000 live births, with a range of about 2 to 12 cases per 10,000 live births.[89] Because similar prevalences are expected in other countries, these figures may be suitable benchmarks for regions lacking accurate historical data.

Microcephaly can occur as a result of fetal brain disruption sequence, a process in which, after relatively normal brain development in early pregnancy, collapse of the fetal skull follows the destruction of fetal brain tissue.[90] The findings of Zika virus RNA in the amniotic fluid of fetuses with microcephaly[40,52,54] and in the brain tissue of fetuses and infants with microcephaly,[55,94,95] as well as the high rates of microcephaly among infants born to mothers with proven antecedent acute Zika virus infection,[69] provide strong evidence linking microcephaly to maternal Zika virus infection. The timing of the Zika virus and microcephaly epidemics in Brazil[96,97] and French Polynesia[41] indicate that the greatest risk of microcephaly is in the first trimester.

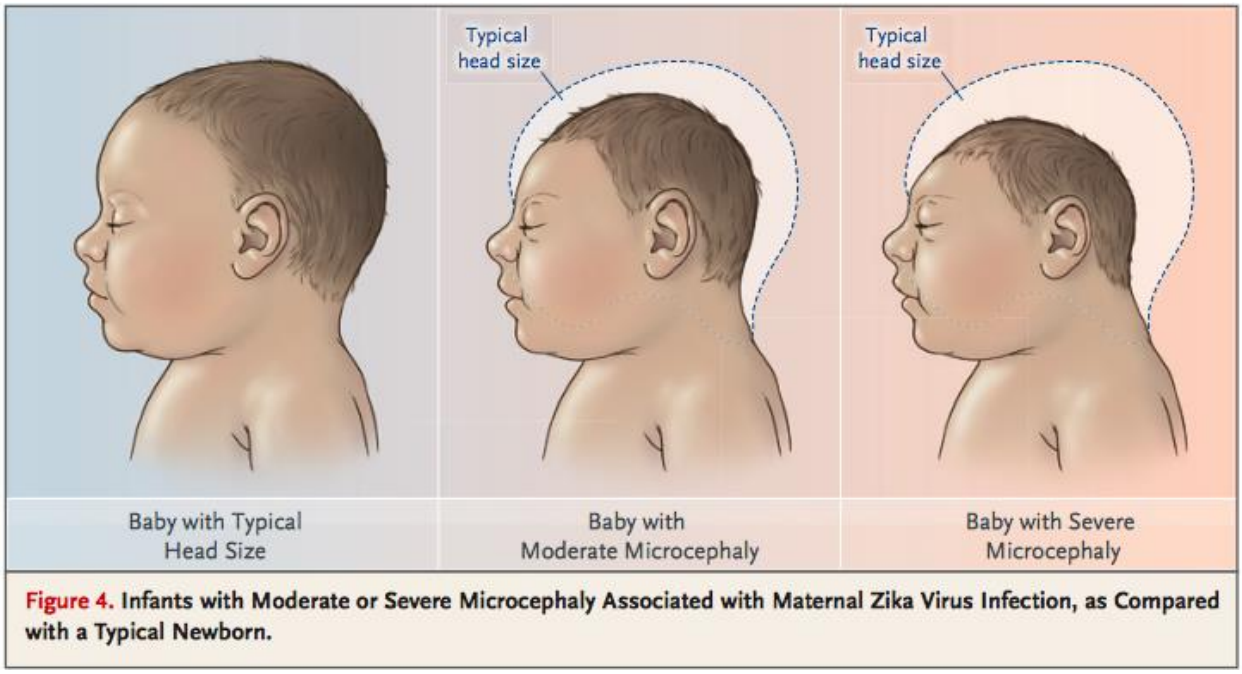


Figure 4. Infants with Moderate or Severe Microcephaly Associated with Maternal Zika Virus Infection, as Compared with a Typical Newborn.

As with the other mosquito-borne flaviviruses, treatment for uncomplicated Zika virus infection focuses on symptoms. No Zika virus vaccine exists; thus, prevention and control measures center on avoiding mosquito bites, reducing sexual transmission, and controlling the mosquito vector.

The Emerging Zika Virus Threat: A Guide for Dermatologists
Author: He et al. (2017)
DOI: 10.1007/s40257-016-0243-z

A 54-year-old man presented to a dermatology clinic in Brazil with a generalized pruritic rash. The rash began acutely 3 days prior to his presentation to the clinic, and was localized initially to his head before spreading to the rest of his body. The patient also experienced photophobia, chills, and conjunctival erythema during this time. He recounts that 1 day prior to the onset of symptoms, he had a headache and diffuse myalgia. He denied fever, diaphoresis, bleeding symptoms, and comorbidities.

Detailed physical exam revealed edema and erythema of the malar region of the face and conjunctival injection (**Figure 1**). There was a macular rash present on his trunk and abdomen (**Figure 2**).

ZIKA

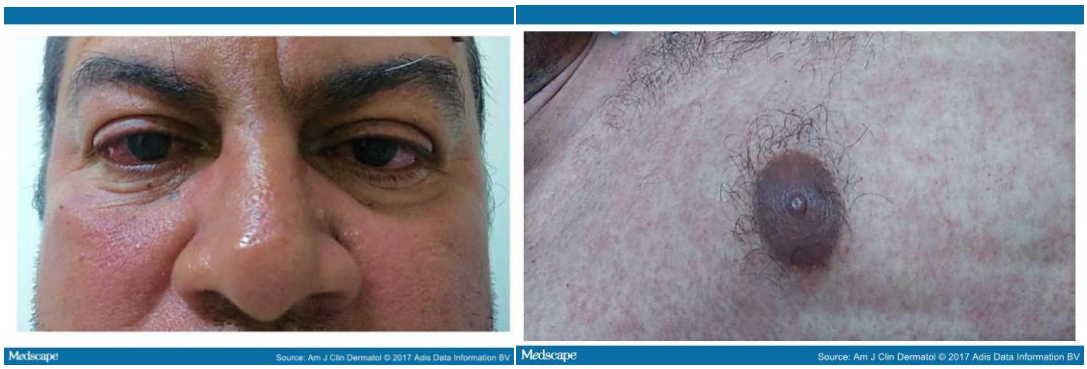


Figure 1 (Left) and **Figure 2 (Right)**.

The patient reported having a neighbor who was ill with a similar presentation a few days before the patient became symptomatic. The patient resided in the metropolitan area of Rio de Janeiro, which is known to be endemic for dengue. He denied recent travel to other areas of the country. As part of the diagnostic work-up, serology tests were performed for dengue, cytomegalovirus, toxoplasmosis, mononucleosis, syphilis and HIV, which were all negative. Real-time polymerase chain reaction (PCR) for dengue and chikungunya also were negative. Due to the current outbreak of Zika virus in Brazil, specific reverse transcription PCR (RT-PCR) for detection of this virus' RNA was performed, which resulted positive for Zika virus.

As there were no critically alarming clinical or laboratory signs, the patient was instructed to increase oral hydration and to return for re-evaluation. On reassessment 2 weeks later in the clinic, the patient reported that his symptoms completely resolved after the sixth day of his illness. The patient was asymptomatic and had no residual signs on examination.

It can be difficult to detect Zika virus clinically, as an estimated 80% of individuals infected with the virus are asymptomatic. Even symptomatic disease is mild, non-specific, and can mimic multiple other diseases and infections, including dengue fever and chikungunya. The rash is a prominent feature of Zika virus infection, and has historically been reported to persist for an average of 6 days, as was the case in our patient.

Zika virus infection damages the testes in mice
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In the presence of the anti-Ifnar1 antibody, high levels of viral RNA (105–108 focus-forming unit (FFU) equivalents per g or ml) and infectious virus (up to 108 plaque-forming units (PFU) per g or ml) were detected in the testis, epididymis and the fluid collected from the epididymis within seven days of infection with either of the two ZIKV strains.

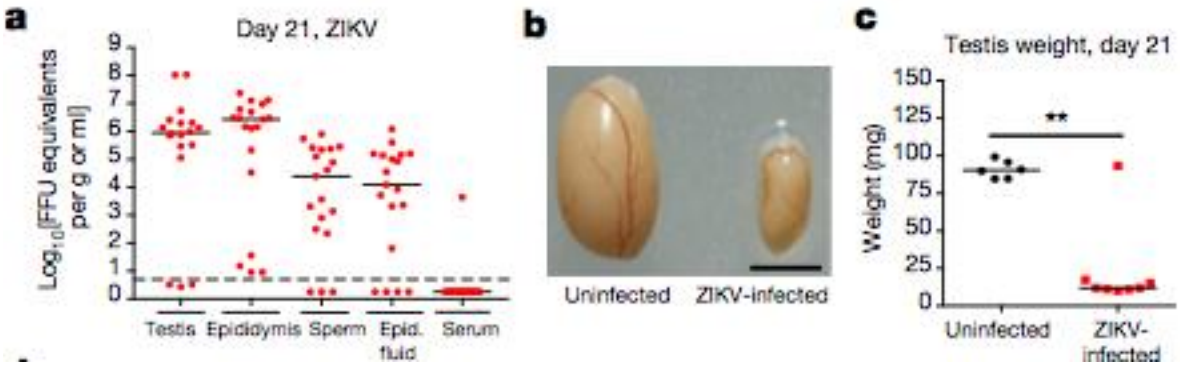

Figure 3 | ZIKV infection of the testis and epididymis at day 21. A. Seven-week-old wild-type mice were treated with 0.5 mg of anti-Ifnar1 at day −1 before subcutaneous inoculation of mouse-adapted ZIKV- Dakar. Tissues and cells were collected at day 21 after infection and analysed for viral RNA by qRT–PCR. Dashed lines indicate limit of detection. Results are pooled from two independent experiments. Bars indicate median values. **B.** A representative image of testes from uninfected and ZIKV-infected mice at day 21; scale bar, 2 mm. **C.** Weight of testes from uninfected and ZIKV-infected mice at day 21.

In most human infections, ZIKV causes a mild febrile illness associated with rash and conjunctivitis. However, severe phenotypes are now appreciated, including Guillain-Barré syndrome[20,21] and congenital abnormalities in fetuses. ZIKV can be transmitted sexually, in contrast to related flaviviruses, as infectious virus persists in the semen of males[23–25] for up to 80 days after symptom onset[2]. Our experiments with mouse-adapted ZIKV-Dakar show that infection causes testicular and epididymal damage in mice that can progress to reductions in key sex hormones, destruction of germ and somatic cells in the testis, and loss of mature sperm and fertility. Sertoli cells may be a key target for ZIKV in the testis, resulting in cell dysfunction, detachment from the basement membrane and dissolution of the BTB. Infiltrating inflammatory cells may amplify destruction of the testicular architecture. Although further studies are required, this pathologic process results in decreased male fertility, at least in mice.