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Evaluating Safety of Utilizing Microorganisms (Bacterial and Fungal Species) as an Alternative for Application of Fertilizers and Pesticides

USAID Assignment on December 5 to 19, Guatemala City, Guatemala Trip Report (January 2022)

Dr. Aliyar Cyrus Fouladkhah, PhD, MS, MPH, CFS, CPH
Faculty Director, Public Health Microbiology Laboratory
Tennessee State University

Photos Courtesy: Adobe Stock, royalty purchased (standard license) by public health microbiology laboratory

It was a great pleasure for me to return to Guatemala for another USAID assignment. Guatemala is a beautiful and culturally-rich country in Central America with agriculture as one of the main contributors to the country's GDP. Although we were in midst of an unprecedented global respiratory infectious disease pandemic/endemic, we were able to make great progress on this two-week assignment. This was in part thanks to the great help and support from USAID F2F offices in Washington and Guatemala and the outstanding safety precautions that were established by USAID offices for the assignment. Because of these, special and sincere appreciation is needed for Ms. Susanna Meyer for her outstanding support and help in harmonizing the events of the program from Washington and to Mr. Jose Eduardo Cano Ozaeta and Mr. Otto Rivera from the Guatemala USAID office.

This assignment was initiated because a local entrepreneur has asked for help and scientific advice for his operation. The operation is a collection of small companies, some are in a pre-revenue stage that is collectively employing around 150 Guatemalans. The founding CEO of these companies has sadly passed away very recently and the son of the original founder, who has inherited these companies, had asked for help and technical support for the operations. Specifically, this assignment was designed to assist an innovative company that grows a selection of microorganisms and sell them to local farmers as products to increase nutrient availability in soil and claim to be efficacious in replacing the use of agricultural chemicals during various stage of production. They are currently producing several products on a low scale and sell them regionally. The company has recently invested in a new facility for the growth and packing of microbial agents. When we attend the assignment in December 2021, the entrepreneur was in process of completing the new processing plant thus this assignment was very timely and of great help for the host company. During the limited discussion that I had with some of the regional farmers, they also expressed tentative interest in the product, primarily since the product, if proven effective, could assist them in avoiding the use of chemicals that could be one of the main barriers for farmers to export the crops since some of the chemicals in use in the country might not have regulatory approval in North America or European union.

In addition to the current assignment, I had a chance to join a local university (UVG) that has recently received a nearly $40M grant from USAID for improving their research infrastructure. During the visit, I had a chance to provide information and exchange idea with a great local faculty member and assist and advise two local doctoral students who were working on plant-based products using microbiology-related topics. Specifically, advice on the biological value of plant- and animal-based proteins and the importance of essential amino acids in the human diet were discussed with the students since they were working on these areas for their doctoral dissertations. Another special highlight of the trip was meeting Mr. Marco Barbie (Owner of Ugy's Super Food). The last time I was in Guatemala in 2018, I had an assignment to assist his company meets the food safety and organic certifications and I was delighted to see that recently, he had been able to secure new organic certification and implement technical information provided to him in last assignment.

Meeting Mr. Marco Barbi, Host of 2018 USAID Assignment Lead by Dr. Aliyar Cyrus Fouladkhah. He was able to receive his organic certification and improve the food safety in his operation based on information and technical assistance provided to him during the previous assignment of F2F USAID in Antigua, Guatemala.

In the United States, I serve as a Process Authority for Association for Food and Drug Officials (AFDO). In that capacity, in 2021, I had a chance to provide technical support and assistance to more than 40 entrepreneurs. With around 67% of these companies being start-up entrepreneurs and around 52% women-owned businesses. Additionally, I have visited and assisted companies and stakeholders in Jamaica, Haiti, Guatemala, Dominica Republic, and in South Africa. Looking back at these experiences, this company is perhaps the one that required and will require in the future the most assistance. During my visit, they were very important, and critical areas of concern that were discussed with the entrepreneur. I also held training sessions on transboundary infectious diseases for 14 members of the company. Excerpts of these slides are accompanied to this document.

The concept of growing microorganisms and using them to reduce or eliminate chemicals in agriculture is a novel and emerging idea and certainly has promise and potential. I sincerely hope the company utilizes these recommendations to improve the safety of their products, showcase whether they are biologically efficacious, eliminate the biological hazards in the facility and

address biosecurity hazards of the products to ensure they can safely sustain and grow the business. Specially, these areas of concerns with recommendations were discussed with the entrepreneur:

Selection of Strains and Their Pathogenicity. Currently, 11 fungal strains and 14 bacterial strains are utilized in the company. Very limited information about these strains are available to the owner and quality control team and considering that they are utilizing extremely high concentrations, safety of the workers in the company and the stakeholders who use the products are of great concern. One of the bacteria they utilize in this company, as an example, is *Pseudomonas fluorescens*. Depending on the strain used, this bacterium could be a potential human pathogen. It is worth mentioning that the company utilizes such extremely high concentrations that there might be no information in the literature about exposure to these extreme concentrations. The following study was shared with the entrepreneurs:

American Society for Microbiology
Clinical Microbiology Reviews
Volume 27, Issue 4, October 2014, Pages 927-948
https://doi.org/10.1128/CMR.00044-14

Review

Microbiology, Genomics, and Clinical Significance of the Pseudomonas fluorescens Species Complex, an Unappreciated Colonizer of Humans

Recommendation: Selecting strains are an extremely important aspect of these products. Existing and future strains would need to be analyzed using culture-independent methods, specifically using whole-genome sequencing followed by bioinformatic analyses to ensure selected strains do not carry genes that are directly associated with human pathogenesis. The conduct of actual analyses is relatively low-cost and could be achieved by paying a few hundred dollars per strain to a sequencing facility. However, bioinformatic analyses and comparing the selected strains with existing library of strains from the same genus and species requires great technical knowledge. At the current state, the products are simply a mysterious and not very known mixture of microorganisms that could potentially have mild to severe pathogenic characteristics

Correction of Product Description and Product Claims. Currently, the products have microbiological claims that are incorrect and exaggerated. For example, a product of the company called BTBP 34SC reports >10^{15} *Bacillus thuringiensis* in addition to 5 other microorganisms each represented at >10 log/mL of the final product. The company did not have documentation on how these values were generated. These numbers are nearly impossible to assimilate from a microbiological perspective.

BIO BTBP 34SC
Larvicida de amplio espectro

Concentración garantizada	% p/v	UFC/mL
Paenibacillus popilliae	7.00	3.56 x 10^{14}
Bacillus thuringiensis	7.00	9.44 x 10^{15}
Beauveria bassiana	5.00	4.40 x 10^{10}
Metarhizium anisopliae	5.00	9.40 x 10^{12}
Lecanicillium lecanii	5.00	2.67 x 10^{9}
Purpureocillium lilacinum	5.00	6.21 x 10^{11}

The products have additional claims that are simply not validated in controlled trials. Limited data are available on selected products and selected crops, however, studies are not conducted in controlled setting and their validation could not be verified. The only aspect that differentiates a product from a "snake oil" type commodity is validation studies conducted using well-designed, adequate powered, and controlled trials.

Recommendation: It is strongly recommended for the company to conduct well-design trials with proper statistical analyses and controls in the design to ensure the claims on the product are evidence-based. Also, it is recommended to use logos of regulatory agencies only if there is prior approval and discussions. In the product presentation material, the logo of the FDA, as an example, was used without any prior approval or relevancy. Claims on food and agricultural products are highly regulated and careful attention is recommended to ensure the scientific validity of the claims and promises of the product.

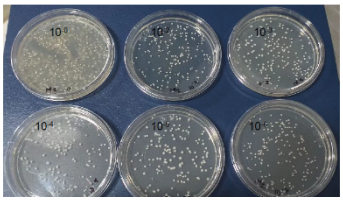
Addressing Occupational Hazards in the Operation. In the current facility, workers are exposed to extremely high levels of fungal and bacterial spore and vegetative cells. Specifically, once spores on grown on trays of sterilized rice, one worker is tasked to manually separate the spores from the rice particles buy using sieves. Although the worker wears protective equipment, that equipment is not sufficient for 100% elimination of airborne microbial agents. Even the N95 masks, by definition, only filter 95% of the airborne particles. This industry has one of the highest levels of exposure to live microorganisms and in the current condition, there are great

occupational hazards. The concern is further augmented as the company has major contamination issues in their operation, based on their own newly conducted internal study. Although the source and type of contaminant are still unknown to the company. During my visit to the company processing plant, there was very strong evidence of airborne contamination that poses the risk to the workers and also several neighbors since the company was located in a residential area and was a domestic house that is converted to the processing plant. The new processing facility, which was under the construction during our visit, had some safety precautions but still does not address the concern of workers' exposure to the microorganisms.

Recommendation: Anytime workers could be exposed to microbial agents at high concentrations, the activity would need to be conducted under a biosafety cabinet or a similar facility design at a larger scale. This image shows an example of a design to ensure that workers are not exposed to respiration of airborne microbial spores. Workers would need to be trained and notified about this occupational hazard and would need to be well-compensated for this hazardous task of handling microorganisms at very high concentrations, rarely seen in any other industry.

Development of Sanitation Standard Operating Procedures (SSOP). Considering that the company is currently experiencing major contamination issues in nearly all their products and considering that the company is currently unaware of the source and genus/species of the contamination, there is a strong need for a major improvement in the sanitation program of the company. The company is growing microbial spores that are extremely resistant to sanitization programs.

Metarhizium anisopliae 021021M						
CONTAMINANTES	DILUCIÓN					
	0	2	3	4	5	6
+++	INC	7.00E+03	1.00E+04	0.00E+00	0.00E+00	0.00E+00



Recommendation: The company would need to develop validated SSOPs to ensure removal of bacterial biofilms, spores, and vegetative cells, fungal colonies, and fungal spores from the production environment. The company was advised on the proper use of SSOPs for their operation.

Further information is provided in the accompanying slides from a teaching session conducted for the company employee.

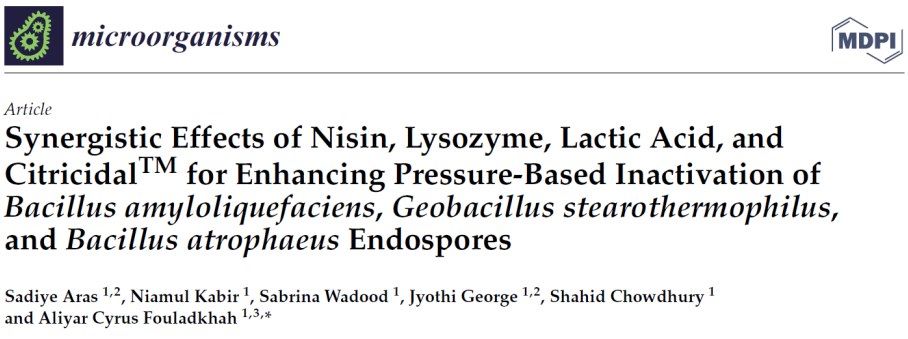
Biosecurity Concerns of the Product. The products of the company have two major biosecurity concerns: (i) the existence of contamination in nearly all products of the company. At the current state, the formulation of the products is a mixture of known and unknown organisms. The unknown organisms could be pathogenic and those that could pose a major biosecurity risk. (ii) The final product contains a growth medium (Nutrient Broth). This simply means that the product could harbor the growth and multiplication of microorganisms if introduced after production and during the shelf-life, making the product a potentially hazardous commodity. For example, if by accident or anthropogenically, *B. anthrax* or typhoidal *Salmonella* serovars are introduced to the product during the shelf-life, these pathogens of public health concern could multiply to levels of concern and when applied in a farm, could contaminate the crops, farm workers, and the soil.

Recommendation: There is no simple solution to this challenge. First, the contamination issue has to be addressed before any further production. This is simply unacceptable and unethical to send a product to the market while the company knows about the extensive contamination of the product. The product would also need to harbor warnings to ensure that users are aware of intrinsic hazards associated with the product. The closure of the product could also be designed in a fashion that reduces the introduction of potential pathogens inside of the product container. The formulation of the product could also be supplemented with agents that are selective and/or differential in nature, minimizing the chance of multiplication of accidentally introduced contaminants. Products could additionally be formulated as a solid fertilizer, eliminating some of the post-production biosecurity hazards.

Improving the Quality of the Products. At the current time, every batch and every container of the product could be substantially different than other batches/containers. There is no stablished method in the company to measure the biological activity of the product- the microorganisms are grown and then simply mixed together and manually packed. Each batch may or may not have all the promised microorganisms and proportion of the microorganisms are simply unknown to the company. The strains are grown and harvested individually but then mixed together so it is unknown how the strains interact with each other and how long they

remain biologically active. Current products are mysterious baches of products and no one in the company exactly knows what the exact microorganisms and proportions are. The microbiological methodologies used are additionally flawed, contributing to lack of consistency and contamination issues. Additionally, there is no established method to determine the amount of vegetative vs. bacterial spores in the products and during production. Finally, it is noteworthy that at the current times, the cultures of the bacterial and fungal strains are left at room temperature and for each batch, a small proportion from the past culture is transferred to the new batch. This could be another major route of contamination as strains require storage at -40 or -80 °C.

Recommendation: These products require the highest level of training in microbiology and infectious diseases to ensure that products are produced without any contamination, remain active during shelf-life, and do not cause biosecurity hazards during and after production. There is need for great improvement in microbiological aspects of the production. Just as an example, currently the company uses the tap water directly for growing the microorganisms that could be the source of contamination since the water and the container used to transfer that to the bioreactor could harbor various microorganisms. The company could simply first autoclave the water and the container and then add the sterilized water to the bioreactor. Additionally, the efficiency of the production could be increased by using proper supplements in the production for example the use of yeast extract could enhance the multiplication of vegetative bacteria and the use of MnSO4 could enhance the production of bacterial spores. The following publication of the public health microbiology laboratory was shared that has best practices for proper preparation and harvesting of spores.



It is also recommended for the company to use centrifugal and mechanical separation for harvesting the microorganisms to remove the growth medium and impurities from the live bacterial/fungal pellets and spores. This could reduce occupational hazards discussed earlier as well.

Establishing Shelf-Life and the Need for Product Re-Application. The shelf-life of the product is currently unknown. The company does not know the biological activity of the product when it leaves the company. The live microbes could be reduced by a substantial number especially since they were placed all at the same container during the packing. The products are not refrigerated, making the need for shelf-life studies even more critical. It is also unknown if the farmers require to use the products more than once. This is a live product and once it is applied, the potentially live microbes could colonize the soil and the product and further multiply, eliminating the need for re-application.

Recommendation: The company's internal staff were trained during this assignment to conduct an internal shelf-life study. This could be achieved by activating each strain aseptically and individually and monitoring the biological activity of the strain over time. Once the shelf stability of individual strains is determined, the same could be conducted using the mixture of the microorganisms. These studies could be conducted at various temperature. Additionally, validation studies, perhaps by working with a university partner, could ensure the need for product reapplication and also validate the biological activity of the product during the shelf-life.

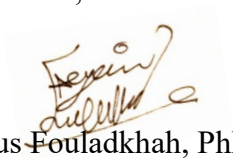
I would suggest the company places a “public health hold,” on products and implement these recommendations very carefully to ensure the products are produced without any occupational hazards and are free of contamination. Additionally, it is recommended to ensure the products have proper and validated claims and have consistency from one batch to the other to ensure the consumers of the product are receiving the commodity they have been promised. It is strongly recommended the company reaches out to individuals with microbiology training to help in implementing these recommendations.

In summary, the company has currently major challenges associated with their operation from major occupational hazards during the production, to existing contamination of the products with currently unknown [to the entrepreneur] microorganisms, to a lack of validated information

about the efficacy of the products. Adherence to these recommendations is strongly recommended for ensuring the safety of the individuals working in the company and those who purchase the product and consumers of the agriculture commodities treated by these promising but currently potentially hazardous products.

With these being said, I would like to sincerely and wholeheartedly thank the great colleagues in Washington and Guatemala F2F offices for their help harmonizing the events of this program and Ms. Crista Rosenberg for technical assistance and translation of material to Spanish. I would also need to greatly thank Dr. Minoo Bagheri for accompanying me on this trip and for providing valuable suggestions and advice for the entrepreneur during this assignment. I commend the host institution for their willingness to learn and encourage them to implement these important recommendations to ensure the safety of their product, their personal, and their consumers.

With best wishes,



Aliyar Cyrus Fouladkhah, PhD, MS, MPH, MACE, CFS, CPH

Associate Professor, Tennessee State University

Faculty Director, Public Health Microbiology Laboratory

Yale School of Public Health Alumnus

The Assignment

Public Health Microbiology Laboratory:
https://publichealthmicrobiology.education/

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To Whom It May Concern,

My name is Susie Meyer and I am the recruiter for the USAID-funded Farmer-to-Farmer program at Partners of the Americas. F2F is a program that promotes economic development and food security abroad by sending volunteer technical specialists from the U.S. to Latin America to provide technical assistance to producers, cooperatives, agribusinesses, NGO's, and educational institutions (referred to as "hosts"). Our program covers all volunteer costs including airfare, lodging, meals, insurance, and any other assignment-related expenses.

The F2F Guatemala office is pleased to invite Dr. Aliyar Cyrus Fouladkhah to support the *Agroindustrias SUCCESSO* a HACCP and Safety Expert from December 5 – 19, 2021.

Please find a description of the needs that Dr. Fouladkhah will address during his 15-day stay in Guatemala:

- SUCCESSO is interested in improving quality in its production processes, HACCP, and strengthening standards to manage safety in the production process of their microorganisms. Dr. Aliyar Fouladkhah will support SUCCESO to analyze their current processes, determine improvement opportunities, identify critical control points, and provide recommendations to improve their current protocols. It is expected that at the end of the assignment, they will have better safety processes, HACCP, and part of the staff trained on how to maintain effective control of the recommendations. This assignment contributes to the broader goals and objectives of the Rural Enterprise Development strategy by strengthening the capacities of production and commercialization of Guatemalan bio inputs, and specifically quality of microorganisms.

Partners of the Americas is following safety protocols in relation to the COVID 19 pandemic and will provide Personal Protective Equipment and maintain social distancing throughout the assignment. All assignments are assessed for risk prior to travel, taking into account the varying levels of COVID-19 throughout the region. Our local Guatemala team and headquarters in Washington, DC are committed and prepared to support Dr. Santamaria throughout the assignment.

Please reach out to our F2F recruitment office at farmertofarmer@partners.net should you have any questions.

Sincerely,
Susie Meyer

Recruitment Office
Farmer-to-Farmer
Economic Development & Health
Partners of the Americas
1424 K Street NW, Suite 700 | Washington, DC 20005
202-637-6206 | farmertofarmer@partners.net | Partners.net/farmer-to-farmer
LinkedIn | Facebook | Twitter | Instagram

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35 YEARS FARMER TO FARMER
The USAID John Ogonowski and Doug Bereuter Farmer-to-Farmer Program

We envision a world where communities and individuals are empowered and sustained through volunteerism and partnerships.
Our Mission is to connect people and organizations across and within borders to serve and change lives.
Partners inspires through our values of Service, Partnership, Impact, Resilience, Respect, Empowerment and Sustainability

The Recommendations

Public Health Microbiology Laboratory:
https://publichealthmicrobiology.education/

FARMER TO FARMER
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Partners Farmer-to-Farmer (F2F) Program – Assignment Report Template

Remote Farmer-to-Farmer volunteers are required to submit (1) recommendations for the host organization and (2) an overall assignment report at the conclusion of their assignment. This template includes both components. Reports are shared with hosts and field staff and are translated as needed.

INSTRUCTIONS

- Topics and Format: Your report should answer all questions below in the text boxes and be within the paragraph range recommended in the prompt. You may include any additional content as annexes attached to your submission email.
- Submission: Please email an electronic copy of your report before the end of your assignment to Bo Maher (rmaher@partners.net) and Susie Meyer (farmertofarmer@partners.net) once you and your field officer have agreed it is finalized.

ASSIGNMENT TITLE (found on Scope of Work):	USAID649_GT_Fouladkhah,A_HACCP

EXECUTIVE SUMMARY

It was a great pleasure for me to return to Guatemala for another USAID assignment. Guatemala is a beautiful and culturally-rich country in Central America with agriculture as one of the main contributors to the country's GDP. Although we were in midst of an unprecedented global respiratory infectious disease pandemic/endemic, we were able to make great progress on this two-week assignment. This was in part thanks to the great help and support from USAID F2F offices in Washington and Guatemala and the outstanding safety precautions that were established by USAID offices for the assignment. Because of these, special and sincere appreciation is needed for Ms. Susanna Meyer for her outstanding support and help in harmonizing the events of the program from Washington and to Mr. Jose Eduardo Cano Ozaeta and Mr. Otto Rivera from the Guatemala USAID office.

BACKGROUND and OBSERVATIONS

In the United States, I serve as a Process Authority for Association for Food and Drug Officials (AFDO). In that capacity, in 2021, I had a chance to provide technical support and assistance to more than 40 entrepreneurs. With around 67% of these companies being start-up entrepreneurs and around 52% women-owned businesses. Additionally, I have visited and assisted companies and stakeholders in Jamaica, Haiti, Guatemala, Dominica Republic, and in South Africa. Looking back at these experiences, this company is perhaps the one that required and will require in the future the most assistance. During my visit, they were very important, and critical areas of concern that were discussed with the entrepreneur. I also held training sessions on transboundary infectious diseases for 14 members of the company. Excerpts of these slides are accompanied to this document.

The concept of growing microorganisms and using them to reduce or eliminate chemicals in agriculture is a novel and emerging idea and certainly has promise and potential. I sincerely hope the company utilizes these recommendations to improve the safety of their products, showcase whether they are biologically efficacious, eliminate the biological hazards in the facility and address biosecurity hazards of the products to ensure they can safely sustain and grow the business.

ACTIVITIES

The host company has recently invested in a new facility for the growth and packing of microbial agents. When we attend the assignment in December 2021, the entrepreneur was in process of completing the new processing plant thus this assignment was very timely and of great help for the host company. During the limited discussion that I had with some of the regional farmers, they also expressed tentative interest in the product, primarily since the product, if proven effective, could assist them in avoiding the use of chemicals that could be one of the main barriers for farmers to export the crops since some of the chemicals in use in the country might not have regulatory approval in North America or European union.
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HOSTS

This assignment was initiated because a local entrepreneur has asked for help and scientific advice for his operation. The operation is a collection of small companies, some are in a pre-revenue stage that is collectively employing around 150 Guatemalans. The founding CEO of these companies has sadly passed away very recently and the son of the original founder, who has inherited these companies, had asked for help and technical support for the operations. Specifically, this assignment was designed to assist an innovative company that grows a selection of microorganisms and sell them to local farmers as products to increase nutrient availability in soil and claim to be efficacious in replacing the use of agricultural chemicals during various stage of production. They are currently producing several products on a low scale and sell them regionally.

RESULTS

(1) Improving in the Selection of Strains and Their Pathogenicity. (2) Correction of Product Description and Product Claims. (3) Addressing Occupational Hazards in the Operation. (4) Development of Sanitation Standard Operating Procedures (SSOP). (5) Addressing Biosecurity Concerns of the Product. (6) Improving the Quality of the Products. (7) Establishing Shelf-Life and the Need for Product Re-Application.

NEXT STEPS and FUTURE VOLUNTEER NEEDS

In summary, the company has currently major challenges associated with their operation from major occupational hazards during the production, to existing contamination of the products with currently unknown [to the entrepreneur] microorganisms, to a lack of validated information about the efficacy of the products. Adherence to these recommendations is strongly recommended for ensuring the safety of the individuals working in the company and those who purchase the product and consumers of the agriculture commodities treated by these promising but currently potentially hazardous products.

PERSONAL REFLECTION

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Volunteer Recommendations Form

Name of Volunteer: Dr. Aliyar Cyrus Fouladkhah
Country of Service: Guatemala Dates of Trip: December 5 to 19, 2021

# of Persons *Formally* Trained[1] – male:	7	
# of Persons *Formally* Trained – female:	8	
# of Persons *Formally* Trained – Non-Binary:	0	
# of Persons *Formally* Trained who are Youth:	0	
# of Persons *Formally* Trained – total:	15	

Please review footnotes for definitions of "persons trained" and "persons directly assisted"

Recommendations Made by the Volunteer:[2]
Please summarize the recommendations you made to the people/groups/organizations you assisted. Details of the recommendations should be included in the trip report – this is a summary table only.

Recommendation	Host	Time frame to implement change
Improving in the Selection of Strains and Their Pathogenicity	**Agroindustrias SUCCESSO**	**One year**
Correction of Product Description and Product Claims.	**Agroindustrias SUCCESSO**	**One year**
Addressing Occupational Hazards in the Operation.	**Agroindustrias SUCCESSO**	**One year**

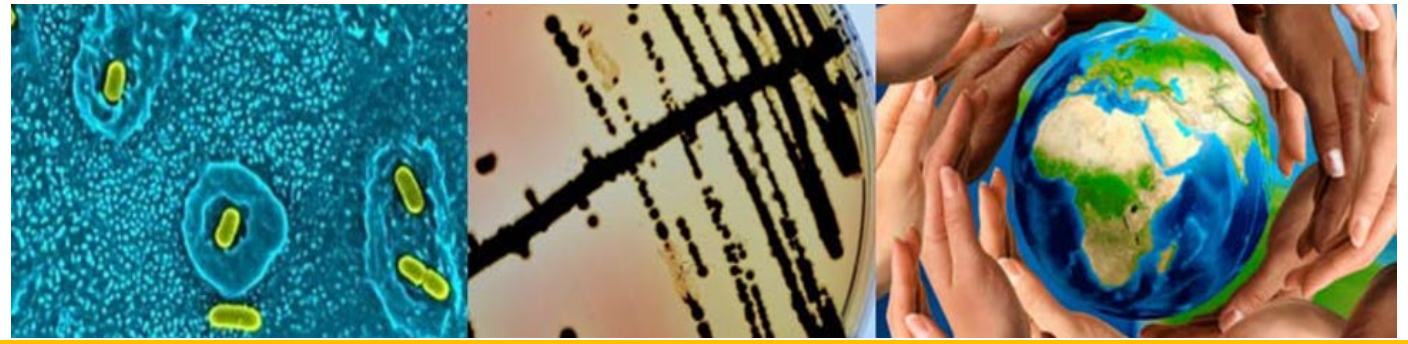
[1] **Persons Formally Trained:** number of persons who received technical/instructional training *in a "formal" setting:* classroom, workshop, institute/university or on-the-job setting with specific learning objectives and outcomes

[2] **Recommendations Made by the Volunteer:** The definition of "recommendation" is quite subjective, but might include an improved procedure, a technological or management innovation, a useful product or marketing tool, etc. Volunteers might make numerous detailed recommendations to a variety of hosts. Recommendations should be written in a way that is clear and measurable. *Please try to limit recommendations to no more than six per host.*

Development of Sanitation Standard Operating Procedures (SSOP). Addressing Biosecurity Concerns of the Product.	**Agroindustrias SUCCESSO**	**One year**
Improving the Quality of the Products.	**Agroindustrias SUCCESSO**	**One year**
Establishing Shelf-Life and the Need for Product Re-Application.	**Agroindustrias SUCCESSO**	**One year**

Excerpts of Teaching Slides

Public Health Microbiology Laboratory:
https://publichealthmicrobiology.education/



Foodborne Diseases of Public Health Importance and Transboundary Diseases

FSMA PC QI Workshop, Guatemala

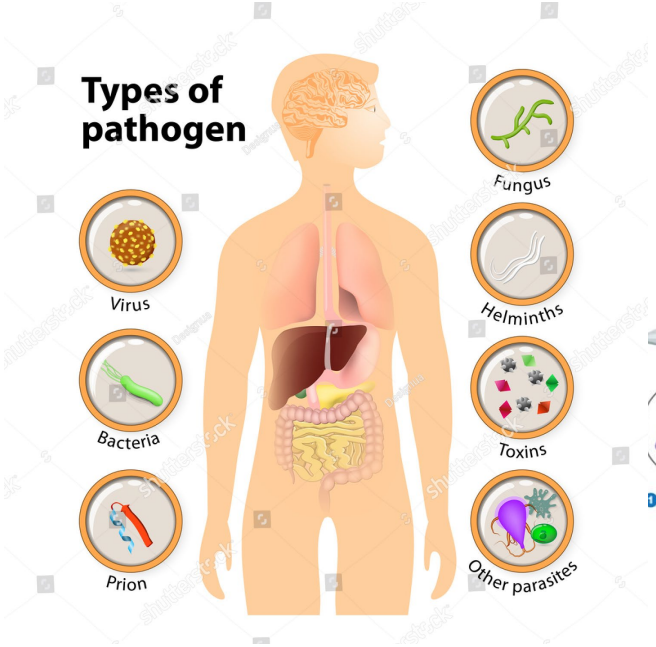
12-7-2021

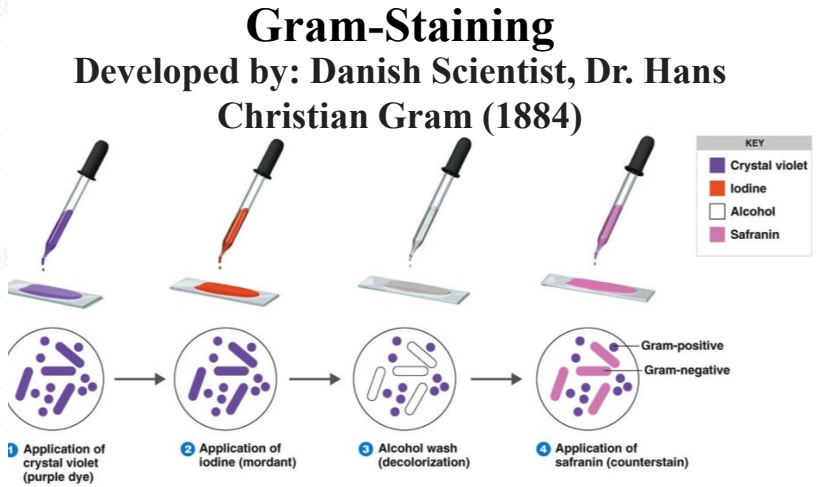
Aliyar Cyrus Fouladkhah, PhD, MS, MPH, CFS, CPH

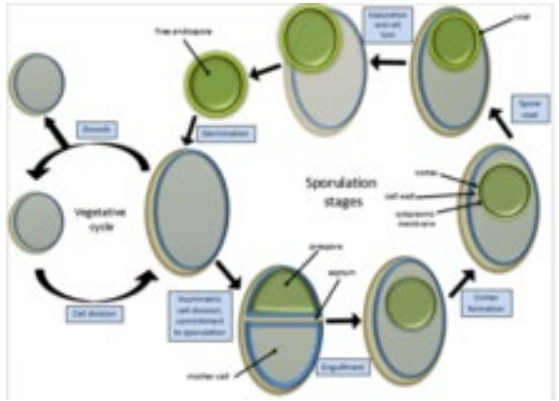
Faculty Director, Public Health Microbiology Laboratory

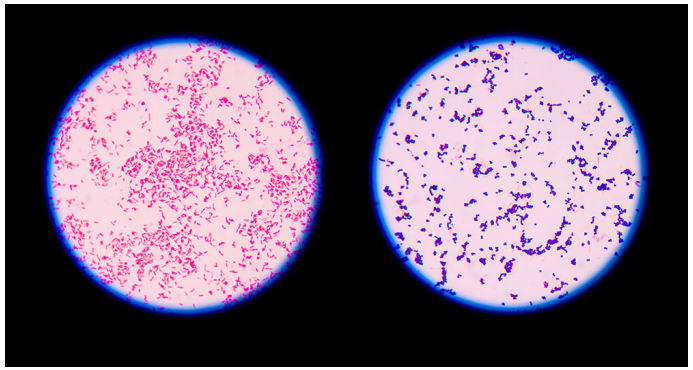
Anthrax

- Causative agent: *Bacillus anthracis*
- A **Gram-positive** and **spore-forming** bacteria
- Can be found as a spore in the **soil worldwide**
- Spores **viable** for **decades in soil**
- **In the US**: Dakotas, northwest Minnesota, Texas, and Nevada
- Common in parts of Africa, Asia, and Middle East
- In Human:
  - Skin
  - Intestine
  - Inhalation
- Animal disease
  - Septicemia and rapid death



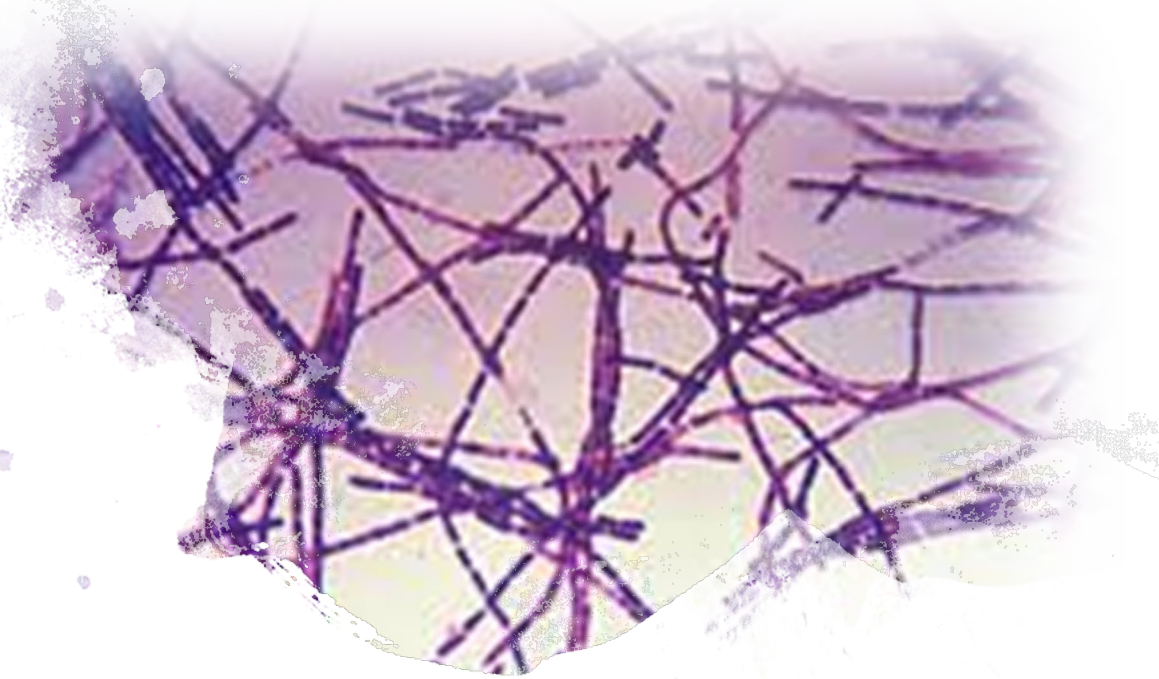






Anthrax

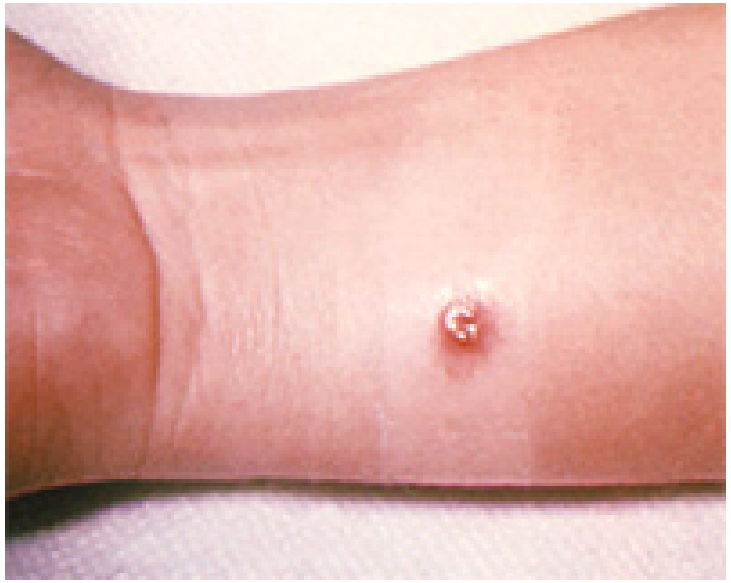
- Spores highly infective
- Remain effective during aerosolization
- Low lethal dose
- High mortality
- Person-to-person transmission rare
- **Symptoms** begin between **one day** and **two months** after the infection



Anthrax- Control and Treatment

- **<u>Four types in human</u>:** Cutaneous (skin); Inhalation; Gastrointestinal; Injection anthrax
- Vaccine for livestock annually to prevent
- Personal Protective Equipment
  - When handling sick animals
- Disinfection:
  - **<u>Sporicidal agents</u>:** 5% formaldehyde, 2% glutaraldehyde, 10% sodium hydroxide
  - **<u>Sterilization</u>:** chlorine dioxide, formaldehyde gas, heating to 121°C for at least 30 minutes
- **Antibiotics:** effective for humans when **prescribed early**

- **Zoonotic Disease**

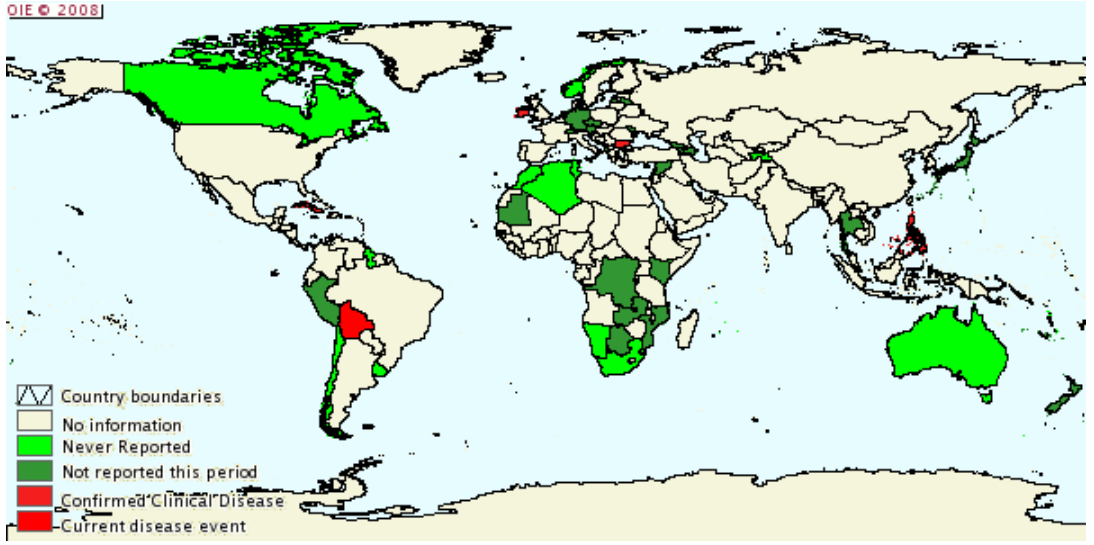


Pseudorabies

- **Contagious** viral diseases from **herpes family**
- Primary concern in domesticated **pigs and feral swine (around 75 million hogs in the United States in 2021)**
- Primarily spread through direct **animal-to-animal** (nose-to-nose)
- **Other mammals**
  - Reproductive
  - Nervous system
- **Humans are not affected**
- Could be a **ubiquitous virus** in some area
- **Eradicated in many countries**
  - Still occurs in parts of world
- Current **USDA Surveillance** to detect any potential case

-Different than rabies that is an important zoonotic diseases.
-Rabies death in the U.S. now < 5 per year
-About 59,000 annually worldwide (>98% from stray dogs)

Source: CDC, 2021



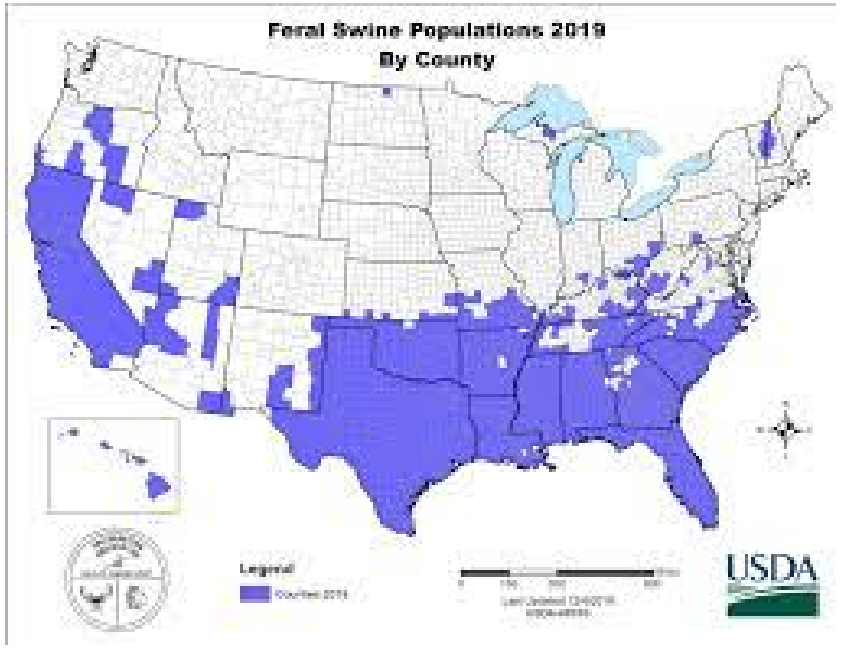
Pseudorabies

- **Transmission:**
  - Direct contact,
  - Reproductive,
  - Aerosol,
  - Ingestion
- **Incubation period: 2-6 days**
- Common symptoms:
  - Neurological
  - Respiratory issues
  - Itching intensively
  - **Stillbirths and abortion**
- **Morbidity and mortality up to 100%**
- **Neonates are particularly susceptible** to the virus

Pseudorabies

- Considered a **reportable disease**
- Could lead to **economic** and **trade restrictions**
- **Treatment** usually **not recommended**
- Current **control practices**:
  - **Depopulation** of the diseased
  - **Test and removal** of carries
  - **Offspring segregation**
- **Vaccine available** in some countries for affected animals

Prevention of Pseudorabies



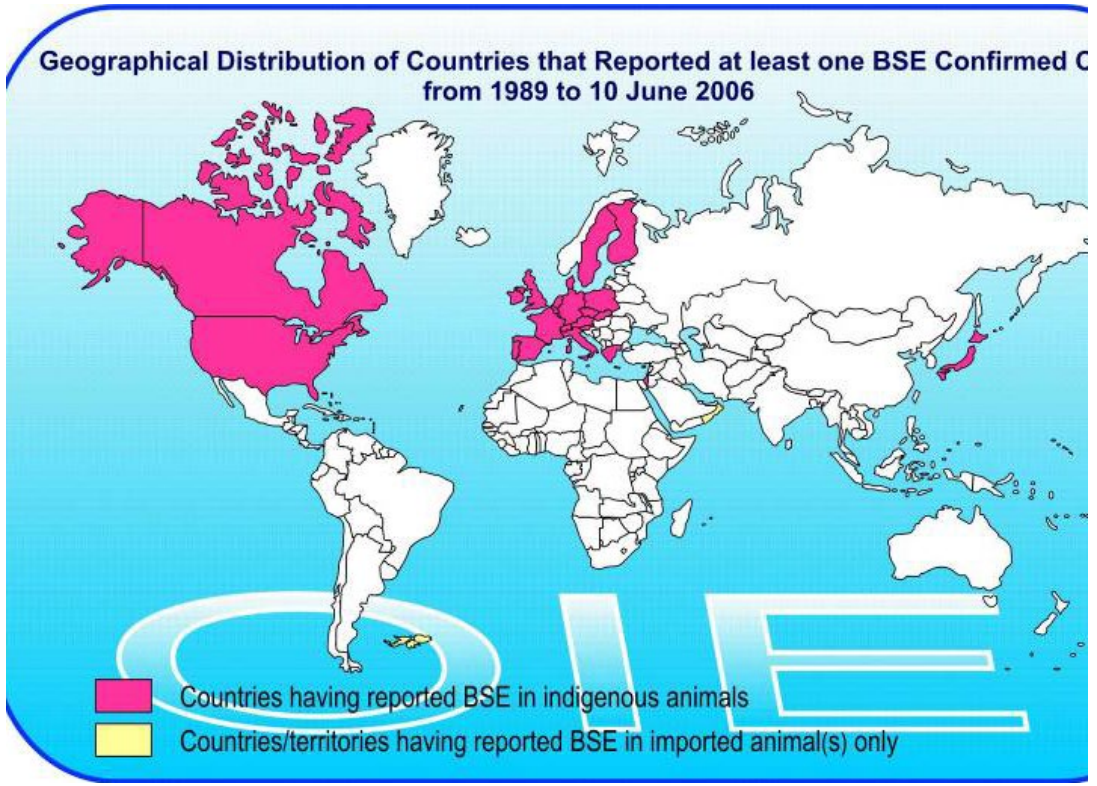
Source: USDA APHIS accessed 2021

- **Isolation:** new or returning animals before entry into the herd
- **Disinfect** vehicles, equipment, premises, footwear
- Separation of pigs and feral swine
- USDA extensive **surveillance program**
  - All 50 states are current free since **April 2008 (commercially)**
  - Feral swine remain as a reservoir of the pathogen

BSE- Bovine Spongiform Encephalopathy

Commonly known as Mad Cow Disease

- Caused by **prions** (infectious protein particles)
- **Cattle and humans** are susceptible
- A neurological disease that could be fatal
- **Transmitted** by:
  - Consumption of **scrapie-infected feed**
  - **Spontaneous mutation**
- Distribution is worldwide



Symptoms of BSE

- In Cattle
  - Incubation period is 2-8 **years**
  - Initial signs are mild and subtle
  - At final stages
    - tremors
    - loss of balance
    - death
- In Humans
  - **Unknown incubation period** (many years to many decades)
  - Neurological signs
  - Depression and schizophrenia-like symptoms
  - Could lead to death

BSE Management

- **Very resistant infectious agent (sanitization very difficult)**
- **Currently no effective treatment or vaccine**
- Prevention:
    - **Surveillance program** and **testing**
    - **Restriction in trade**
    - **Animal feed regulation** (bone meals and mammalian products)
- Outbreak in 2001-2002 in United Kingdom: Cost the industry 3.7 billion Euro

Brucellosis

- Caused by bacteria (several species)

(Genus *Brucella* e.g. *B. melitensis, B. abortus, B. suis, and B. canis)*

  - **Highly infectious (N95 or KN95 mask during farm visits?)**
  - Easily **aerosolized**
- **Transmission**:
  - Ingestion
  - Inhalation
  - Direct contact
- **Signs in animal**:
  - Reproductive complications
- **Signs in humans**:
  - Cyclic fever and
  - Flu-like symptoms

Brucellosis- Treatment & Prevention

- Treatment: long-term antibiotics (Problem: Diversity of causative agents)
- Prevention:
    - **Vaccination** of calves
    - **Minimizing exposure to wildlife**
    - **Segregation of infected animals**
    - **Disinfection of environment**
- **No vaccine available for human**

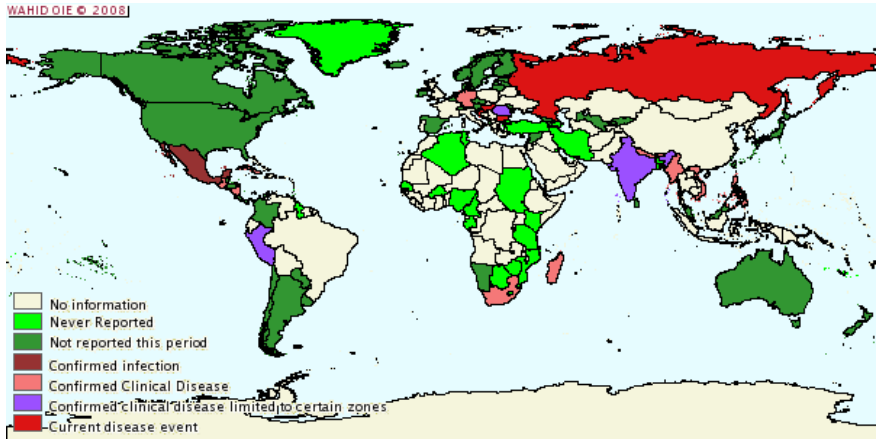
Main infection source for human:

- Contaminated milk, cheese, and ice-creams
- Handling farm animals (glove, goggle, secondary outfit +mask?)
- Hunting Activities

Classical Swine Fever

- **Viral Disease** and very contagious and economically significant
- Disturbed worldwide
- **Spread through**:
  - Ingestion of virus
  - Direct contact
  - Aerosol
  - Insects (vector-bone disease)
- Feeding swine **untreated food wastes** containing infected pork scraps can cause infection
- (**By Product, Animal Food Regulation, FSMA**)

Source, USDA APHIS, accessed 2021



Classical Swine Fever

- Incubation period is 2-14 days
- **Clinical signs variable** depending on:
  - **Strain of virus**
  - **Susceptibility and genetic makeup of the pig**
- Signs very similar to many swine diseases
- Signs could be acute to asymptomatic
- Main symptoms:
  - fever
  - weakness
  - anorexia
  - **purplish discoloration** of skin of ears, inner thighs
  - Could cause death

Classical Swine Fever

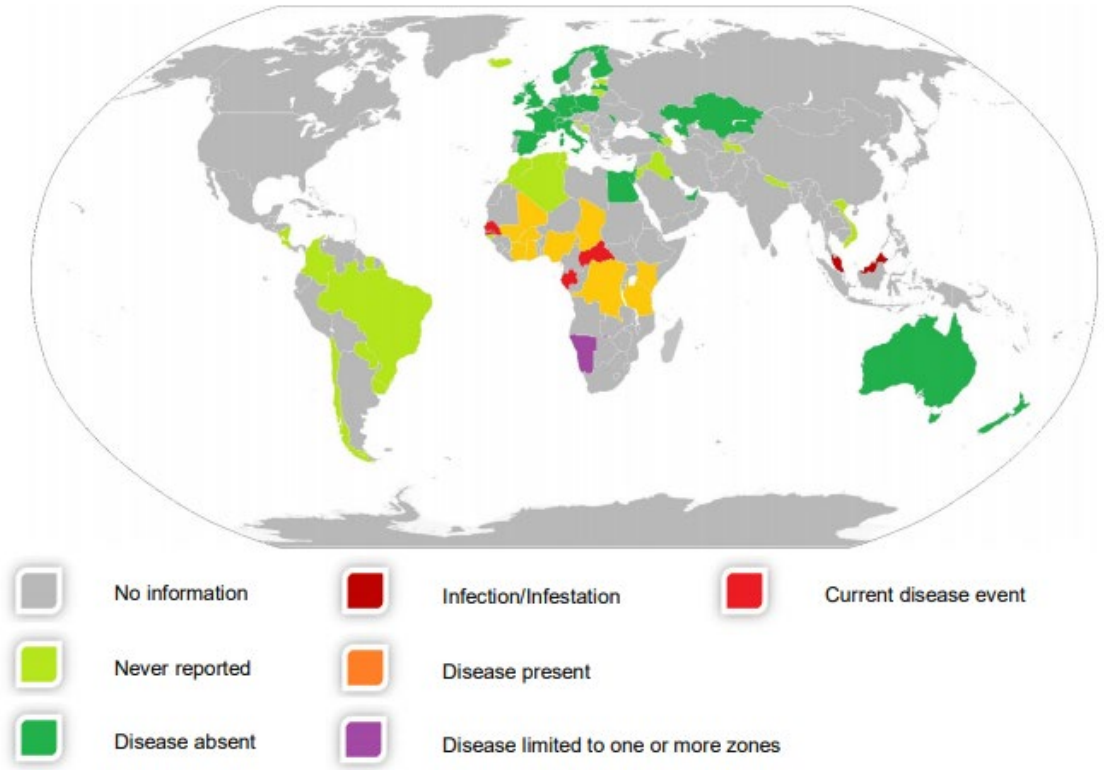
- **Not a zoonotic diseases**
- Could cause **100% mortality** in **swine herds**
- Could cause **import/export restrictions** and economical losses
- Controlled by:
  - **Quarantine**
  - **Slaughter**
  - **Vaccine in endemic area**

Was **eradicated** from the U.S. in 1978

Producers obligated to **report sporadic cases** to USDA APHIS point of contact

Contagious Bovine Pleuropneumonia (CBPP)

- **Bacterial** diseases
- Mainly a concern in **Cattle** (**93 million** head inventory in the U.S. with about **31 million as beef cattle**, as of 2021)
- Also, a concern in **Buffalo, bison, yak, water buffalo**
- Transmission by:
  - Aerosol in **close contact**
  - **Transplacental**
  - Direct contact
    - **Saliva**
    - **Urine**
    - **Fetal fluids**
- **Eradication** had been successful in **UK and Australia**



Sources, USDA APHIS accessed in 2021

CBPP

- Incubation period is 20-123 days
- Respiratory signs
  - Cough
  - Broad stance
- **Morbidity** could be as high 100% in a herd with close contact
- **Mortality** could range from 10 to 70%
- **Vaccine available** in endemic counties
- Human are immune, **not a zoonotic disease**

Equine Encephalitis Viruses

- Three viruses:
  - Eastern (EEE)
  - Western (WEE)
  - Venezuelan (VEE)
- Transmitted by mosquitoes (**vector-borne disease**)
- **Birds** could be **asymptomatic carrier**
- **Clinical signs** in human and Equids (Horses, mules, donkeys)
  - No to mild signs to
  - Flu-like illness
  - Encephalitis in small proportions
  - **Can also infect a wide range of animals including:** mammals, birds, reptiles, and amphibians

Equine Encephalitis Viruses

- The viruses are **very unstable** in environment
- **Supportive care** is the only current treatment
- **Vaccine are available** for Equine
- **Vaccine for human very expensive** primarily for:
  - Researchers
  - Public health workers with enhanced exposure

  - **Travel Clinics for International Travel**

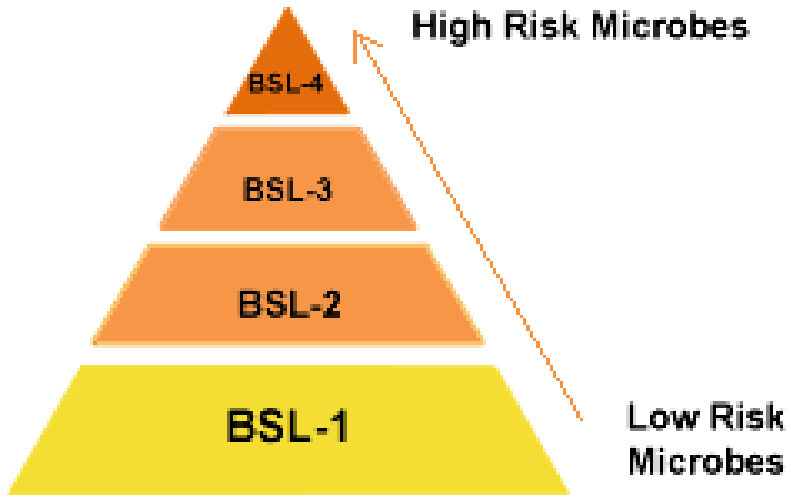
Hendra Virus

- Viral disease **consider as emerging** (**first observed in Australia**)
- Natural infections had been **reported only** in:
  - Horses
  - Humans (first reported in 1994, very rare and under-reported)
- Current transmission by:
  - Fruit bats
  - **Bodily fluids** and **urine** of those infected
- Clinical signs in horses
  - Sudden respiratory signs
  - Nasal discharge
  - Fever
  - Encephalitis
  - Sudden death
- Clinical signs in Humans
  - Flu-like illness
  - respiratory complications
  - **Highly fatal in human, could be as high as 2 in 3 cases**

Hendra Virus

- Little is known about pathogen
- **People at risk:**
  - Those occupational or recreational **exposure to horses**
  - Those **living close to "Flying fox" bats** (genus *Pteropus*)
  - **Researchers**
- Highest level of security (**CDC biosafety level 4**) needed for studying the pathogen (around 4 labs in the US and <50 in the words, as of 2021 [US has about 1,500 BSL3)
- Could cause high mortality in humans
- Currently no treatment option is available

(Great topic for term paper)



Main Bacterial Pathogens Associated with Animal and Human Health Diseases

Emerging pathogens

Diversity, moving towards *"fitness" and Emerging Pathogens*

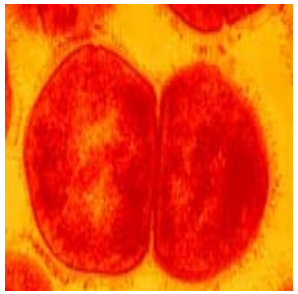
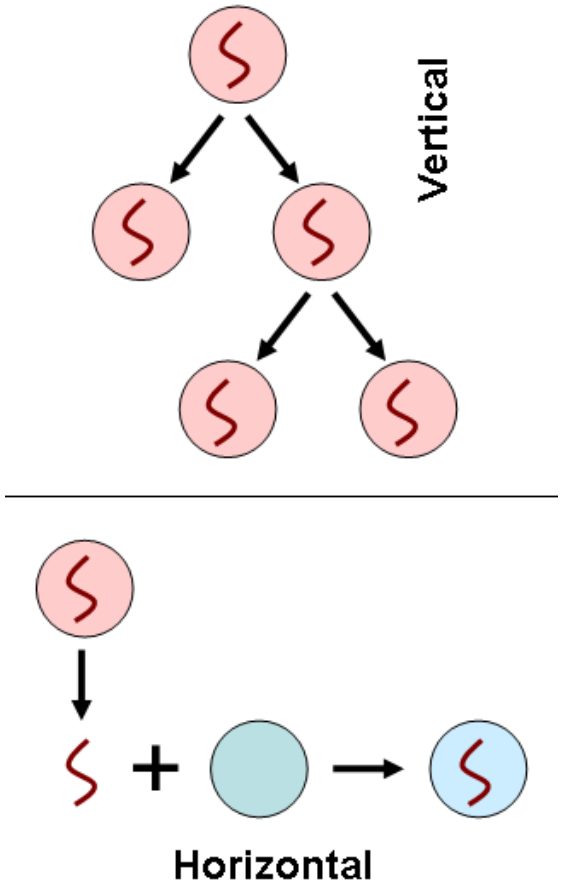
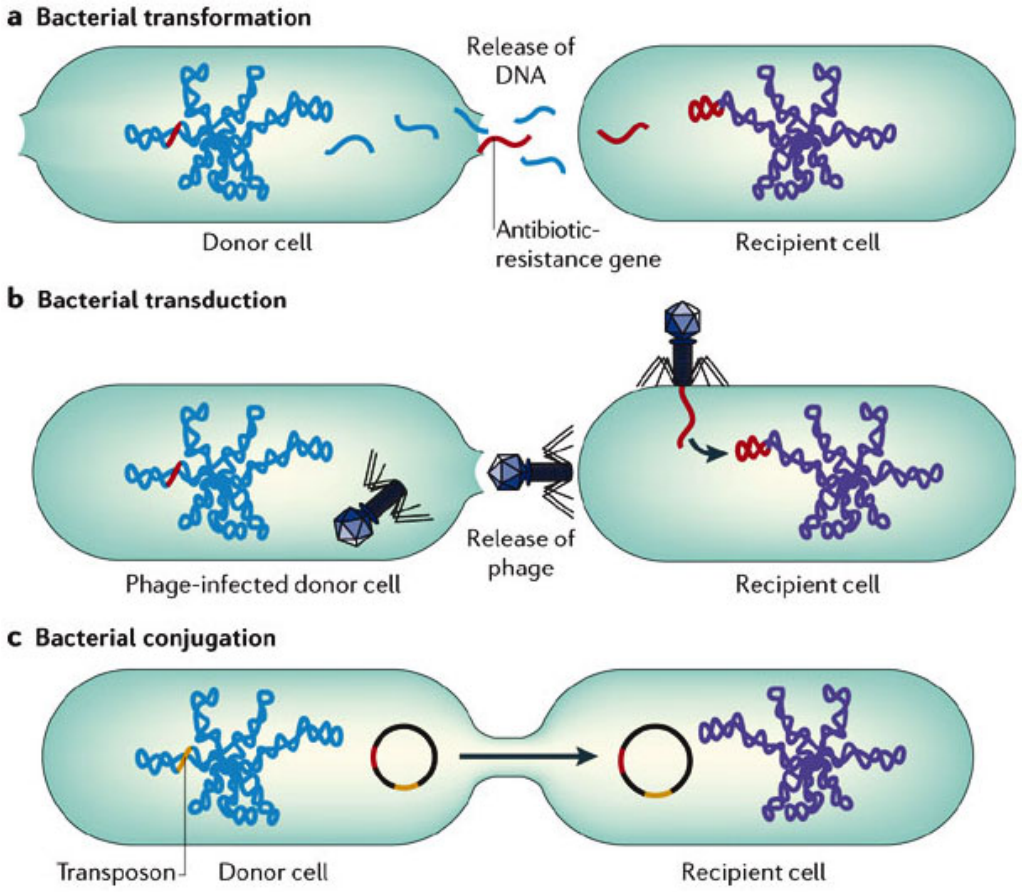
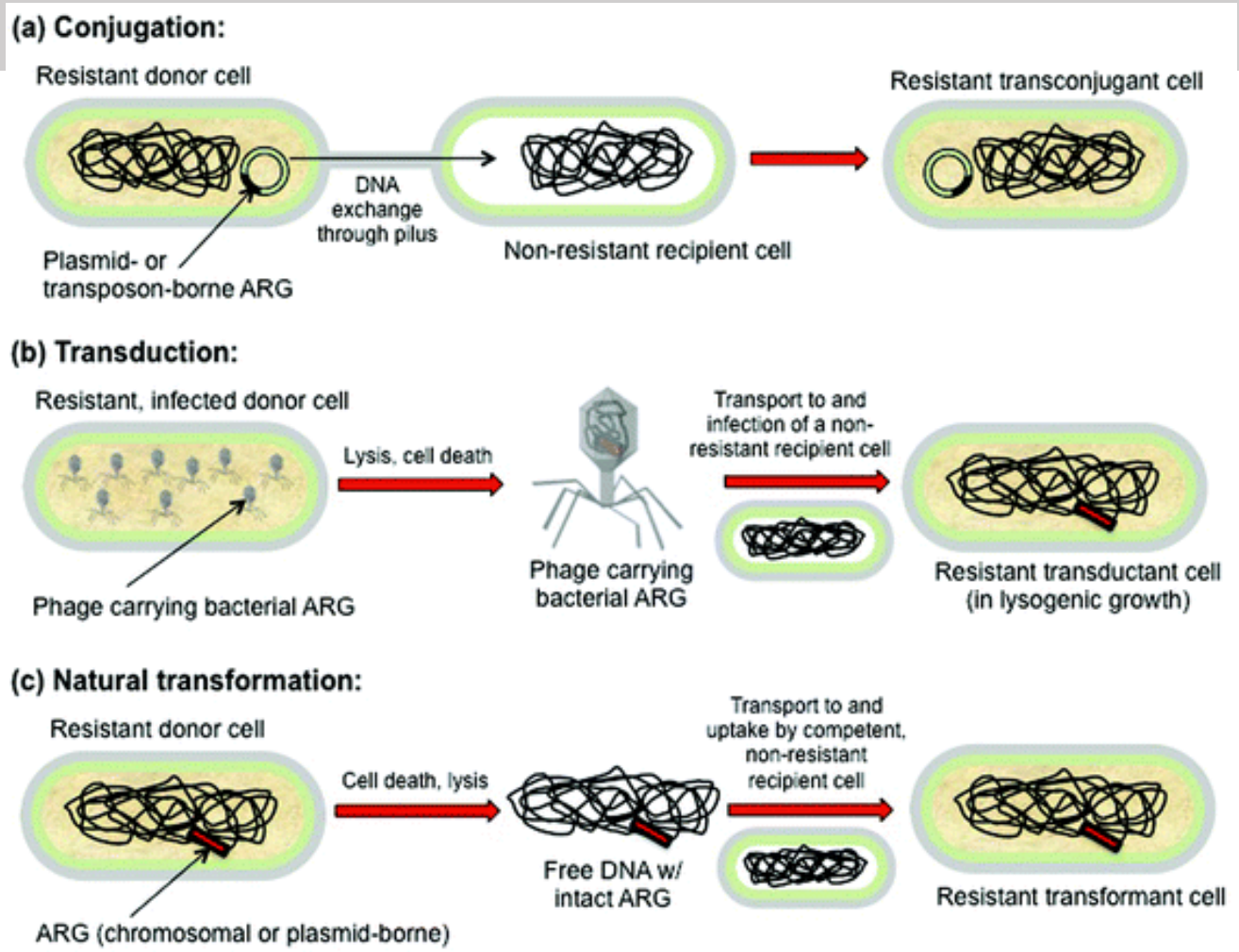


Photo Courtesy:
http://www.daviddarling.info/encyclopedia/B/binary_fission.html





Horizontal Gene Transfer



Donn, 2012

Planktonic cells and Biofilm Communities

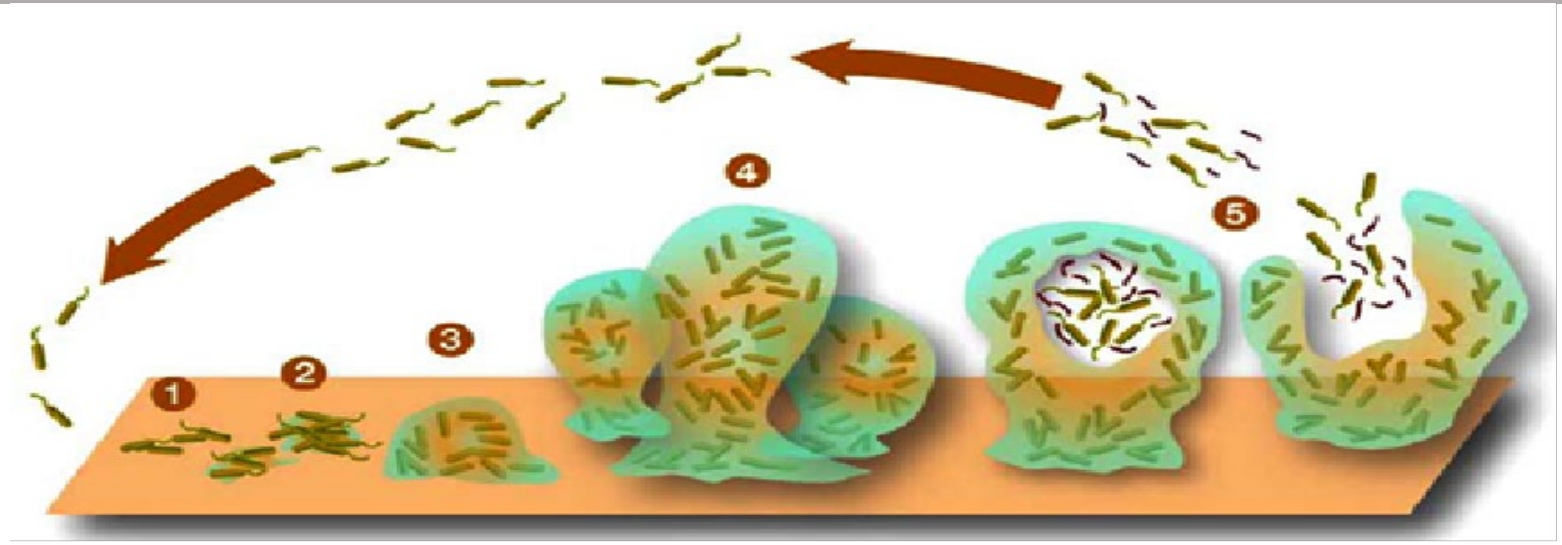


Photo Courtesy: http://prometheus.matse.illinois.edu/glossary/biofilms/

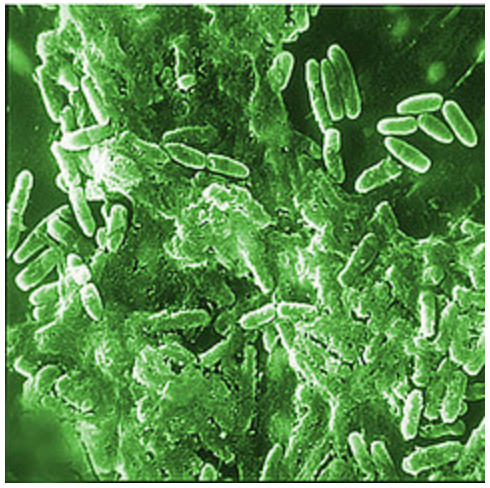


Photo Courtesy: http://micro-writers.egybio.net/blog/?tag=antibiotic-resistance

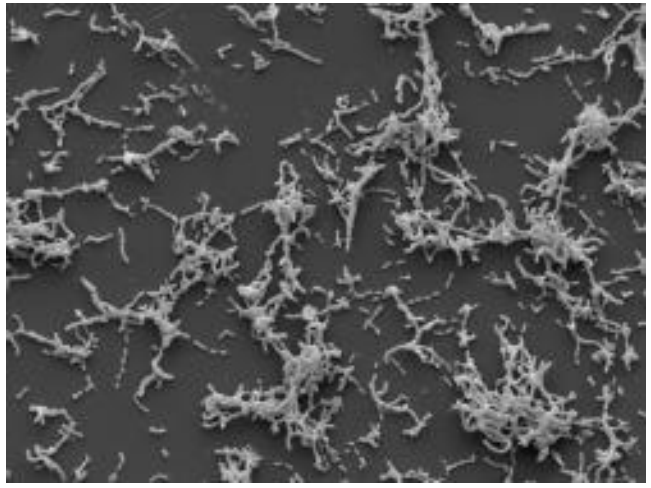


Photo Courtesy: http://www.ifenergy.com/50226711/boosting_microbial_fuel_cells_with_biofilm.php

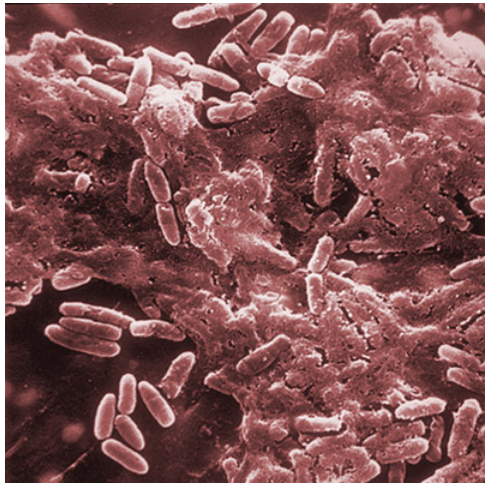


Photo Courtesy: http://www.microbiologybytes.com/blog/category/biofilms/

Quorum Sensing and Biofilm formation

Shiga toxin-Producing E. coli and antibiotics treatment

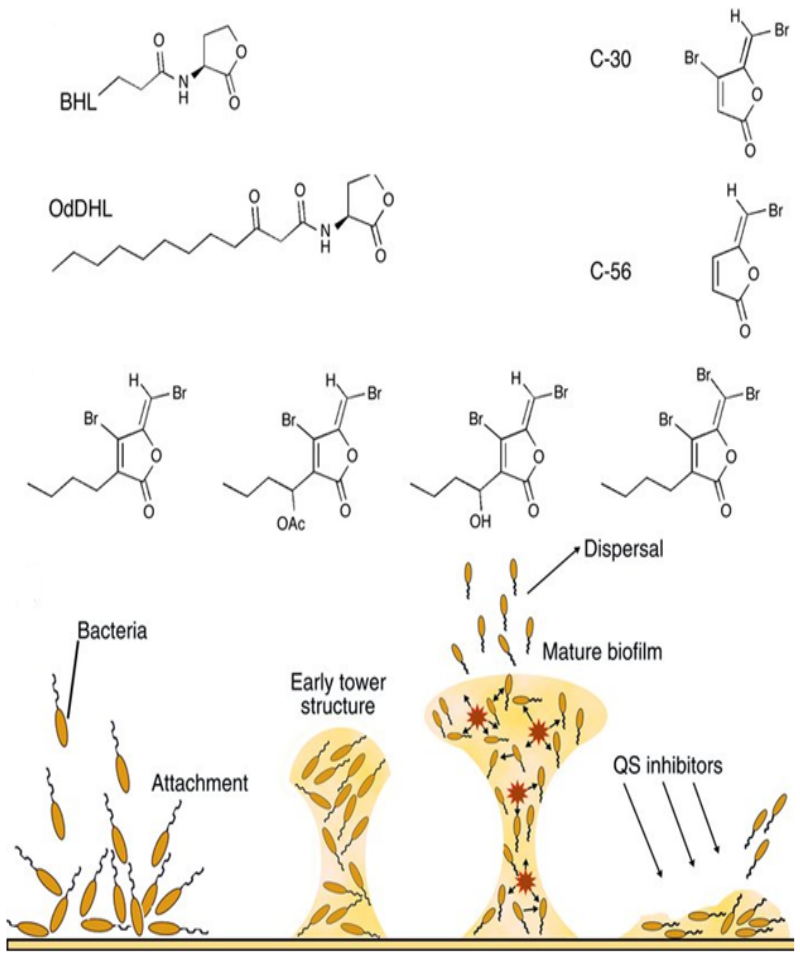


Photo Courtesy: http://www.jci.org/articles/view/20074/figure/2

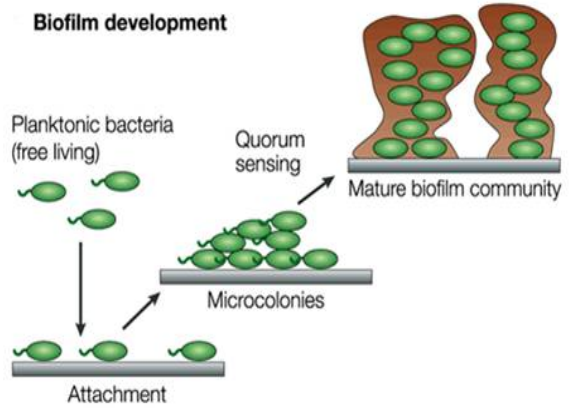


Photo Courtesy: http://labrat.fieldofscience.com/2010/07/quorum-sensing-and-biofilms.html

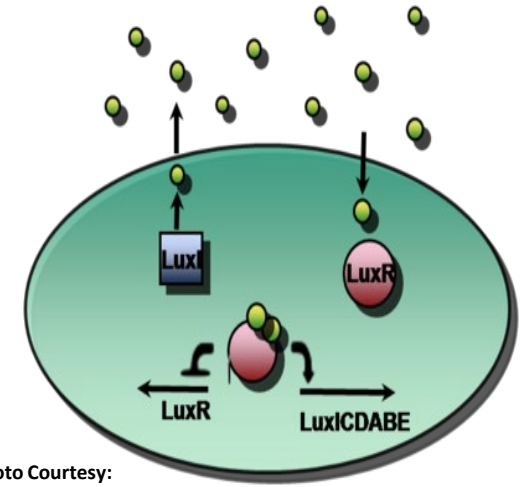


Photo Courtesy:
http://2009.igem.org/Team:Aberdeen_Scotland/WetLab/quorumsensing

Infectious Diseases in Animals and Human is a Moving Target…

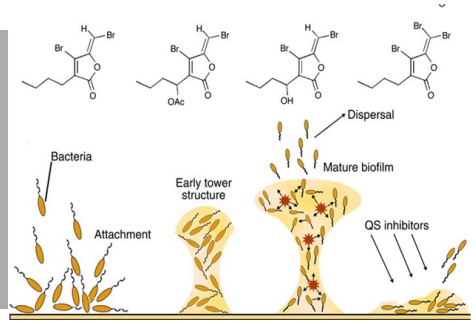


Photo Courtesy: http://www.jci.org/articles/view/20074/figure/2

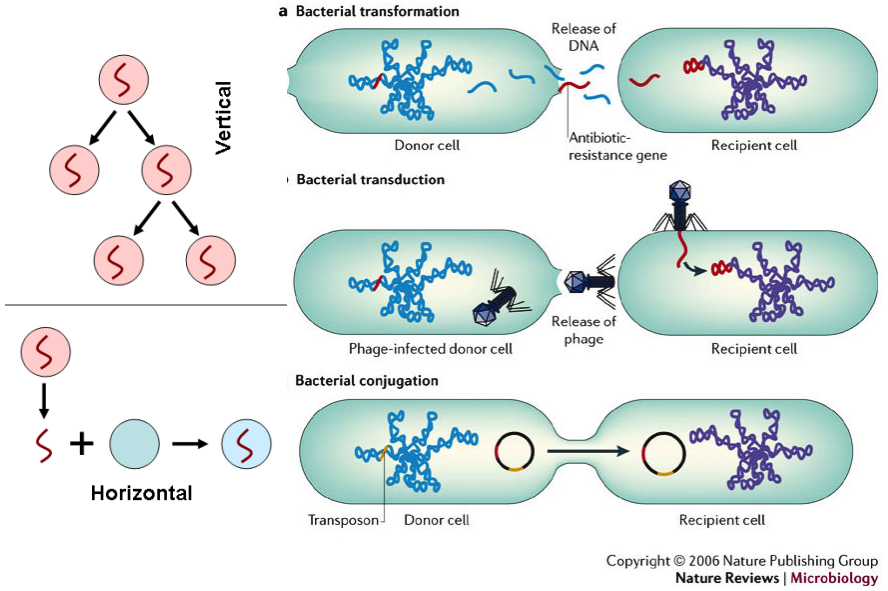
- It is estimated only 1% of microbial community has been identified.
- Currently **etiological agent** of **80.3% of foodborne illnesses**, **56.2% of hospitalization**, and **55.5% of deaths** remain unknown.

"Emerging" Pathogens:

- Vertical and horizontal gene transfer spores and biofilm formation
- Quorum sensing and cell to cell communication

"It is the microbes who will have the last word."

-Louis Pasteur



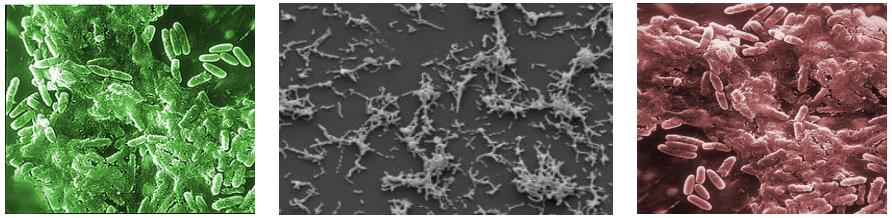


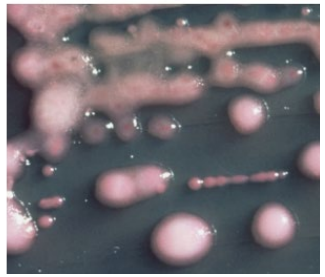
Photo Courtesy: http://www.microbiologybytes.com/blog/category/biofilms/
http://www.ifenergy.com/50226711/boosting_microbial_fuel_cells_with_biofilm.php
http://micro-writers.egybio.net/blog/?tag=antibiotic-resistance

A superbug resistant to every available antibiotic in the U.S. kills Nevada woman

79424

EMAIL

BY HELEN BRANSWELL, STAT *January 13, 2017 a*



Rare strain of E. coli strikes across Canada: source unknown

BY NEWS DESK | JANUARY 14, 2017

A dozen cases of E. coli O121 have been confirmed in three Canadian provinces, according to matching genetic fingerprint data, but the source of the outbreak has not yet been identified.

The Public Health Agency of Canada reports four of the rare O121 cases were confirmed in British Columbia, four in Saskatchewan and four in Newfoundland and Labrador. The illness onset dates were in November and December of 2016.

Four of the victims have been hospitalized. Those individuals have recovered or are in the process of recovery. An investigation to determine the source of the relatively rare E. coli O121 contamination is ongoing.

The Public Health Agency of Canada says improper handling of ground meat and eating raw ground meat are two of the most common sources of E. coli illnesses. Other common sources are contaminated raw fruits

New outbreaks linked to Italian style meats; one third of patients hospitalized

By Coral Beach on August 24, 2021

Inspectors are looking for specific sources of two new Salmonella outbreaks that have been associated with Italian-style meats. Three dozen people from 17 states have been confirmed infected so far.

One of the recalled products

Almost 200 sick in UK-wide Salmonella outbreak

By Joe Whitworth on August 24, 2021

Nearly 200 people across the United Kingdom are part of a Salmonella outbreak linked to pork scratching products.

Photo illustration

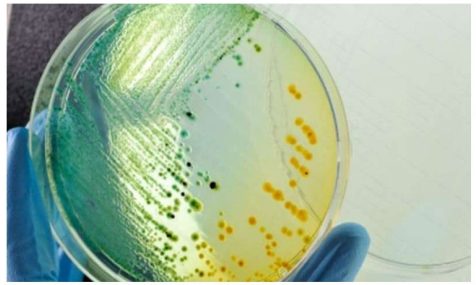
CDC says outbreak traced to raw clover sprouts has come to an end

By News Desk on April 23, 2020

The Food and Drug Administration's investigation of an outbreak of E. coli 0103 in

AUGUST 23, 2019

Multidrug-resistant salmonella outbreak characterized

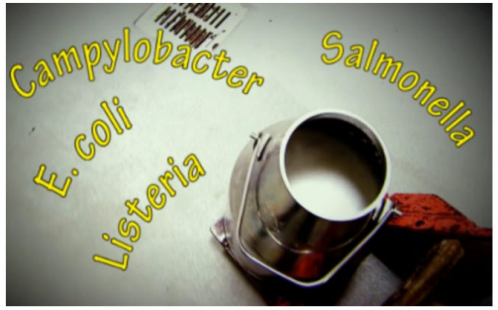


(HealthDay)—A recent multidrug-resistant (MDR) *Salmonella enterica* serotype Newport outbreak, affecting patients in 32 states, was associated with soft cheese and beef consumption, according to a report published in the Aug. 23 issue of the U.S. Centers for Disease Control and Prevention *Morbidity and Mortality Weekly Report*.

Eat Smart chopped salad kit recalled in Canada over Listeria concerns

By News Desk on August 25, 2021

Curation Foods is recalling Eat Smart brand "Asian Sesame (Sésame asiatique) Chopped Salad Kit" because of possible Listeria monocytogenes contamination.



Raw goat milk recalled because of positive test for Campylobacter

By News Desk on August 25, 2021

State officials in California have ordered a recall and quarantine of certain raw goat milk because tests have shown it to be contaminated with Campylobacter.

Epidemiology of Foodborne Diseases

- **Based on data from 1990s:** *(Mead et al., 1999)*

76 million illnesses, 323,000 hospitalizations, **5,200 deaths** in the United States.

- **More recent estimates show:** *(Scallan et al., 2011)*

47.8 million illnesses, 127,839 hospitalizations, and more than **3,037** deaths in the United States.

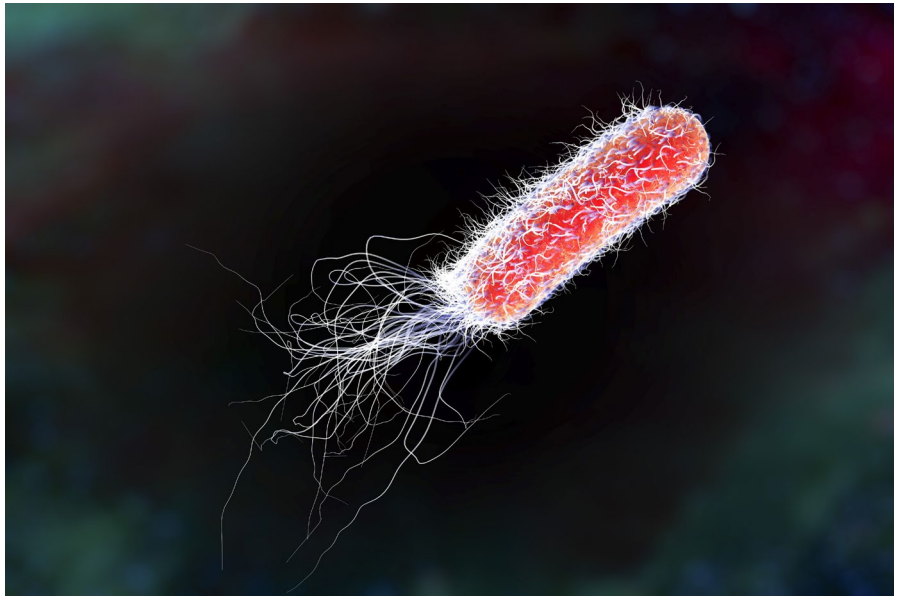
- 9.4 million illnesses, 55,961 hospitalizations, and 1,351 deaths are cause by 31 known foodborne agents.
- In addition to consumer insecurity, foodborne diseases cause around $**77.7 billion** for losses in productivity and economical losses.
- Approximately 30% of population are especially “at risk” for foodborne diseases (The **YOPI**’s: The young, the old, Pregnant, and Immunocompromised)

Significant foodborne pathogens…
based on Mead et al., 1999 and Scallan et al., 2011 studies

- **Leading etiological agents for illnesses**: *Norovirus* (58%), Nontyphoidal *Salmonella* serovars (11%), *Clostridium perfringens* (10%), and *Campylobacter* spp (9%).
- **Leading etiological agents for hospitalization:** Nontyphoidal *Salmonella* serovars (35%), *Norovirus (26%), Campylobacter* spp (15%), and *Toxoplasma gondii* (8%).
- **Leading etiological agents for death:** Nontyphoidal *Salmonella* serovars (28%), *T. gondii* (24*%), Listeria monocytogenes* (19%), and *Norovirus (11%).*

Signs and Symptoms of Foodborne Diseases

- Mild illness (no medical care sought)
- **Guillain–Barré syndrome** (*Campylobacter* and *Salmonella*)
- **Post-infectious irritable bowel syndrome** (*Campylobacter* and *Salmonella*)
- **Reactive arthritis** (*Campylobacter* and *Salmonella*)
- **Haemolytic uraemic syndrome** (*E. coli* O157)
- **End-stage renal disease** (*E. coli* O157)
- Death

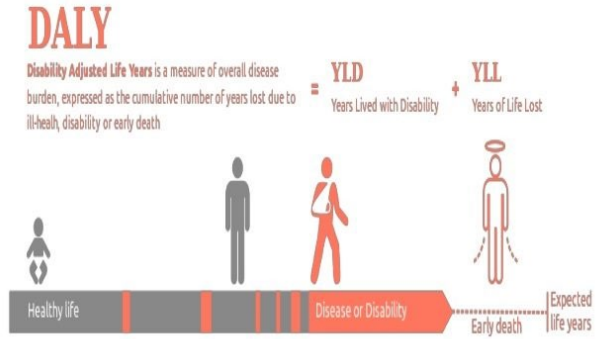


Significant Foodborne Pathogens of Public Health Concern: Considering DALY and QALY *(Scallan et al., 2015)*

- **Disability Adjusted Life Year *(DALY)***. *Loss of life and health due to illness compared with 'perfect' health*

- Non-typhoidal *Salmonella* (329000)
- Toxoplasma (32700)
- *Campylobacter* (22500)
- Norovirus (9900)
- *Listeria monocytogenes* (8800)
- *Clostridium perfringens* (4000)
- *Escherichia coli* O157 (1200)

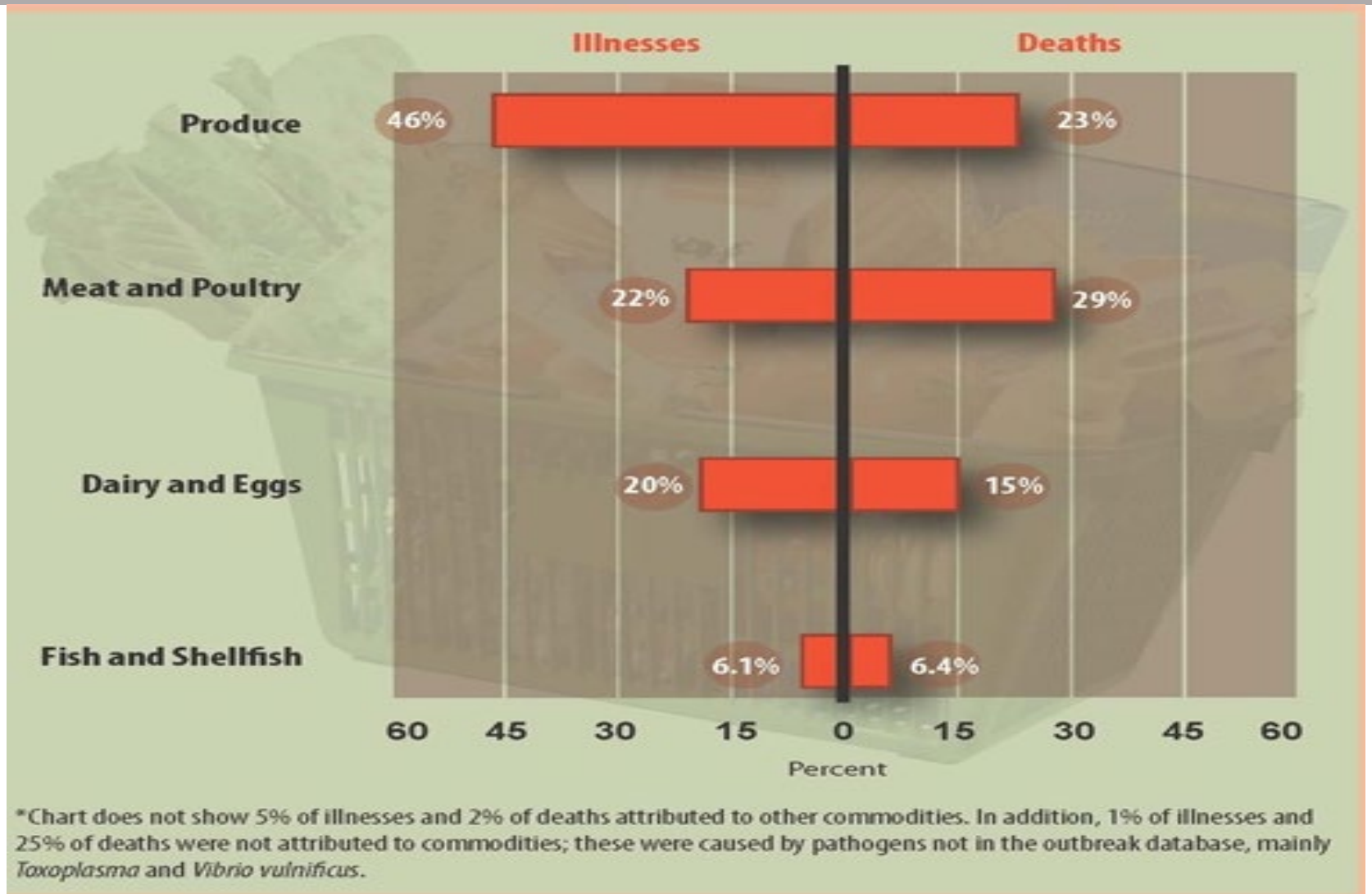
62% bacterial agents; 29% parasitic agents; 9% viral agents



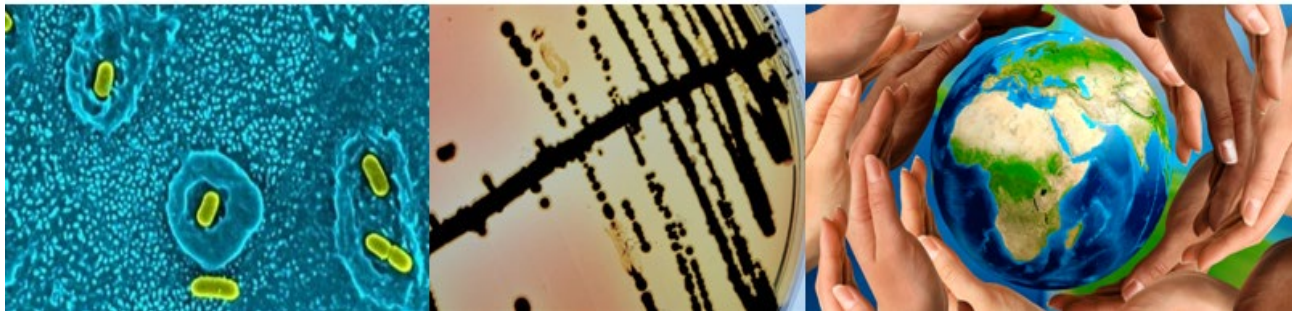
- Mild illness (no medical care sought)
- **Guillain–Barré syndrome** (*Campylobacter* and *Salmonella*)
- **Post-infectious irritable bowel syndrome** (*Campylobacter* and *Salmonella*)
- **Reactive arthritis** (*Campylobacter* and *Salmonella*)
- **Haemolytic uraemic syndrome** (*E. coli* O157)
- **End-stage renal disease** (*E. coli* O157)
- Death

CDC Estimates of Food Safety Burden

http://www.cdc.gov/foodborneburden/attribution-image.html#foodborne-illnesses



Foodborne Pathogens of Public Health Concerns *>200 foodborne diseases*



- *Salmonella* serovars
- *Staphylococcus aureus*
- *Campylobacter* spp.
- *Bacillus cereus*
- Shiga Toxin-Producing *Escherichia coli* (STEC)
- *Vibrio* spp.
- *Yersinia enterocolitica*
- *Streptococcus* spp.
- *Shigella* spp.
- *Listeria monocytogenes*
- *Mycobacterium bovis*
- *Cronobacter sakazakii*

Salmonella serovars

- **Annual illness (death): 1,027,561 (378) in American adults and children**
- **Infection** causes nausea, vomiting, diarrhea, fever, headache
- **Primary sources**: Intestinal tract of people and animals
- **Transmitted by** meat, poultry, eggs, raw milk, unpasteurized juice, many other foods (nuts, spices, produce, chocolate, flour) [Low-moisture environment]
- **Contributing factors**: cross-contamination, undercooked food, poor agricultural practices

Growth parameters	Minimum	Optimum	Maximum
Temperature	41°F (5.2°C)	95-109°F (35-43°C)	115°F (46.2°C)
pH	3.7	7-7.5	9.5
a_W	0.94	0.99	>0.99
Other	Non-spore former		
Atmosphere	Facultative - grows with or without oxygen		

Sources: ICMSF 1995 and Bad Bug Book 2nd edition, Scallan et al., 2011, and FSPCA

Salmonella serovars

- **Carriers**: **Reptiles** (turtles, lizards, and snakes); **Amphibians** (frogs and toads); **Poultry** (chicks, chickens, ducklings, ducks, geese, and turkeys); **Other birds** (parakeets, parrots, and wild birds); **Rodents** (mice, rats, hamsters, and guinea pigs); Other **small mammals** (hedgehogs); **Farm animals** (goats, calves, cows, sheep, and pigs); **Dogs; Cats; Horses. [Pretty much ubiquitous!]**
- **Dogs and cats** that become ill from *Salmonella* infection generally will have **diarrhea** that may contain blood or mucus
- Some cats do not have diarrhea, but will have a **decreased appetite, fever, and excess salivation**.

Prevention:

- **Minimizing direct contact**, **washing hands**, and **cleaning up after the pets** could minimize the risk of transmission from infected animals to human.

Salmonella serovars

CDC Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™

Salmonella Outbreaks Linked to Backyard Poultry

Investigation Notice

Posted July 23, 2021

One in four sick people is a child younger than 5 years. Don't let young children touch chicks, ducklings, or other backyard poultry.

Fast Facts

- Illnesses: 672 (198 new)
- Hospitalizations: 157 (54 new)
- Deaths: 2 (1 new)
- States: 47 (1 new)
- Investigation status: Active

FDA U.S. FOOD & DRUG ADMINISTRATION

Pet Turtles: Cute But Commonly Contaminated with Salmonella

Turtles commonly carry bacteria on their outer skin and shell surfaces that can make people very ill. Geckos and bearded dragons can also infect people.

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Staphylococcus aureus

Foodborne Diseases
- Infection
- Intoxication
- Toxico-infection

- **Annual illness (death): 241,148 (6) Americans every year**
- Both causes infection and toxico-infection
- Produces **heat stable toxins** after extensive growth
- **Primary sources**: Boils, nasal passages and skin (around 20% positive on nasal passage, >10% hands)
- **Transmitted** by recontaminated **cooked foods**, and foods with high salt or high sugar (**Gram-positive, poor competitor**)
- **Contributing factors**: Recontamination and **time/temperature abuse**

Growth parameters	Minimum		Optimum		Maximum	
	Growth	Toxin	Growth	Toxin	Growth	Toxin
Temperature	45°F (7°C)	50°F (10°C)	99°F (37°C)	104-113°F (40-45°C)	122°F (50°C)	118°F (48°C)
pH	4	4	6-7	7-8	10	9.8
a_W	0.83	0.85	0.98		>0.99	
Other	Poor competitor, non-sporeformer					
Atmosphere	Facultative – grows with or without oxygen, but slower without					

Sources: ICMSF 1995 and Bad Bug Book 2nd edition, Scallan et al. 2011, and FSPCA

Campylobacter spp.

- **Annual illness (death): 845,024(76)**
- Infection causes diarrhea, and potential nerve damage
- **Primary sources**: Intestinal tract of animals
- **Transmitted** by raw poultry, raw milk products, contaminated water, poultry (dump tank, nearly 80%). Relatively high infective dose
- **Contributing factor**: cross contamination and undercooking

Growth parameters	Minimum	Optimum	Maximum
Temperature	86°F (30°C)	108-109°F (42-43°C)	113°F (45°C)
pH	4.9	6.5-7.5	9.5
a_W	>0.987	0.997	-
Other	Non-spore former		
Atmosphere	3-5% oxygen optimum		

Sources: ICMSF 1995 and Bad Bug Book 2nd edition and FSPCA

Bacillus cereus

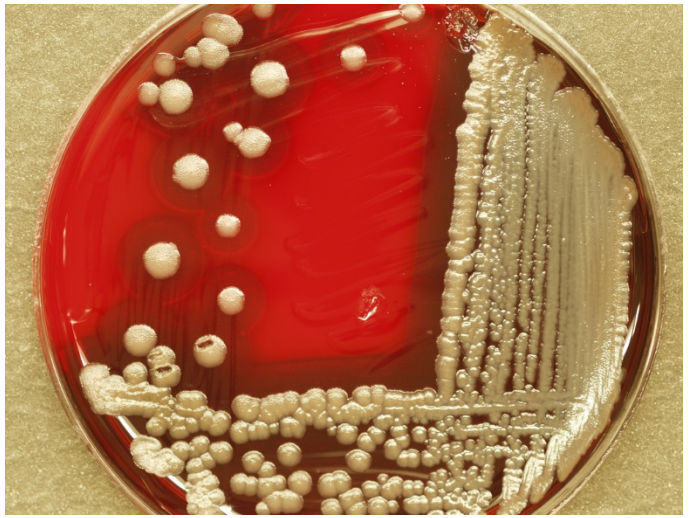
- **Annual illness (death): 63,400 (0)**
- Produces spores and toxins and extensive growth is required for illness
- **Primary source**: soil and GI track
- **Transmitted** by: rice and starchy foods, meats, vegetables, milk products, sauces
- Contributing factors: **temperature abuse**

Growth parameters	Minimum	Optimum	Maximum
Temperature	39°F (4°C)	82-95° F (28-35°C)	131°F (55°C)
pH	4.3	6.0-7.0	9.3
a_W	0.92	-	-
Other	Spore former; toxin is heat stable		
Atmosphere	Facultative – grows with or without oxygen		

Sources: Seafood Hazards Guide, ICMSF 1995, Bad Bug Book, Scallan et al. 2011, and FSOCA

Bacillus cereus

- Some studies indicate the bacterium could behave as an agent of mammary gland **infection in cows** and **goats** thus causing **mastitis.**
- Cases of **food poisoning in dogs and cats** had also been reported, although not very frequent in nature.
- Many agricultural animals carry the **bacterium in their intestinal area** without symptoms.



Shiga Toxin-Producing *Escherichia coli* (STEC)

- **Annual illness (death): 176,152 (20)**
- **Notable outbreak:** 1992-1993 outbreak in pacific northwest- Very important regulatory status (adulterant)
- **Infection causes** bloody diarrhea, and sometimes kidney failure and death [HUS in kids]
- **Primary sources**: Intestinal tract of ruminant animals (e.g., cows, sheep)
- **Transmitted** by raw and undercooked beef, poultry, leafy greens, and unpasteurized milk and juices
- **Contributing factors**: poor GAP, inadequate heating, and person-to-person

Growth parameters	Minimum	Optimum	Maximum
Temperature	**44°F (6.5°C)**	95-104°F (35-40°C)	121°F (49.4°C)
pH	4	6-7	10
a_w	0.95	0.995	-
Other	Non-spore forming		
Atmosphere	Facultative - grows with or without oxygen		

Sources: ICMSF 1995 and Bad Bug Book 2nd edition,Scallan et al. 2011, and FSPCA

Shiga Toxin-Producing *Escherichia coli* (STEC)

- **Animals that can spread *E. coli* O157 to humans include**:

  -Cattle, especially calves (As high 80% in some herds),

  [Concentrated and genetic similarity]

  -Goats

  -Sheep

  -Deer

- *E. coli* infection very common in **cats and puppies younger than one week**.
- **Colostrum**, plays a pivotal role in protecting a newborn the animal's undeveloped immune system against *E. coli* infection.
- As high as **80% of agricultural animals could carry various serogroups of shiga-toxigenic *E. coli*** without having symptoms

Vibrio spp.

- Causing about **80,000 illness and 100 death** annually in the United States.
- **Infection symptoms** vary depending on strain, ranging from diarrhea to high fever
- Vibrio is a **halophilic bacterium** and is a major concern in aquaculture industry
- **Primary sources**: Salt water environments and seafood
- Requires salt to reproduce (halophile)

Growth parameters	Minimum	Optimum	Maximum
Temperature	41°F (5°C)	99°F (37°C)	114°F (45.3°C)
pH	4.8	7.8-8.6	11
a_W	0.94	0.98	0.996 (10% NaCl)
Other	Non-sporeformer, requires salt		
Atmosphere	Facultative - grows with or without oxygen		

Sources: Seafood Hazards Guide 2011, ICMSF 1995 and Bad Bug Book 2nd edition

Yersinia enterocolitica

- *Not a reportable disease, no statistics available*
- **Infection causes** abdominal pain, fever and diarrhea. May mimic appendicitis.
- **Primary sources**: Raw pork, raw milk
- **Contributing factors**: Cross-contamination between raw pork products and RTE foods

Growth parameters	Minimum	Optimum	Maximum
Temperature	30°F (-1.3°C)	77-99°F (25-37°C)	108°F (42°C)
pH	4.2	7.2	10
a_w	0.945	-	-
Other	Non-spore former, raw milk in fridge?		
Atmosphere	Facultative - grows with or without oxygen		

Sources: Seafood Hazards Guide, ICMSF 1995, and Bad Bug Book

Foodborne Streptococcus spp.

- *Not a reportable disease, no statistics available (not part of active surveillance data of CDC)*
- **Infection causes** sore throat, tonsillitis and fever
- **Primary sources**: **Infected sites of humans and animals**, **raw milk**
- **Contributing factors**: Infected **workers handling food** and **consumption of raw milk** or **meat products**.
- **Symptoms**: meningitis, sepsis, and pneumonia (>200,000 sepsis cases per year, not foodborne)
- **Found in**: cattle, horses, dogs, rabbits, guinea pigs and mice
- Important cause of mastitis in cows.

Growth parameters	Minimum	Optimum	Maximum
Temperature	50°F (10°C)	99°F (37°C)	<113°F (<45°C)
pH	4.8-5.3	7	>9.3
%NaCl (salt)	-	-	<6.5
Other	Non-sporeformer		
Atmosphere	Facultative - grows with or without oxygen		

Sources: Seafood Hazards Guide, ICMSF 1995, and Bad Bug Book

Shigella spp.

- **Infection in humans causes** diarrhea, which may be watery to bloody. The infection is also known as dysentery [Taxonomy similar to *Salmonella* serovars]
- **Primary sources**: Human and Animal intestinal tract
- **Transmitted** by **fecal contamination** from contaminated **water** or **infected food**.
- **Clinical signs are rare in dogs and cats**, mostly mild diarrhea.
- Many species such dogs, cats, rodents and nonhuman primates could **carry the pathogen asymptomatically.**

Growth parameters	Minimum	Optimum	Maximum
Temperature	43°F (**6.1°C**)	-	117°F (47.1°C)
pH	4.8	-	9.3
a_W	0.96	-	-
Other	Non-spore former		
Atmosphere	Facultative - grows with or without oxygen		

Sources: Seafood Hazards Guide 2011, ICMSF 1995 and Bad Bug Book 2nd edition

Mycobacterium bovis

- **Infection** causing respiratory symptoms and **tuberculosis**
- **Primary sources**: Cattle and raw milk
- **Other source**: bison, elk, and deer.
- **Contributing factors**: Lack of milk **pasteurization** and exposure to aerosols from infected animals
- **Grows very slowly and under reduced oxygen (microaerophilic)**
- The **US has nearly eliminated** *M. bovis* infection from cattle, over **one million animal is tested** for the bacterium by inspectors. [USDA FSIS]
- *M. bovis* can be found in **wild animals** such as bison, elk, and deer; uninfected cattle that come into contact with these wild animals can become infected.

Source: CDC at https://www.cdc.gov/tb/publications/factsheets/general/mbovis.pdf

Listeria monocytogenes

- **Infection causes** severe illness in susceptible people – mortality 15-30%
- **Primary sources**: Occurs widely in agriculture (soil, plants and water) –(Important during pregnancy)
- **Transmitted by**: Refrigerated RTE foods that support growth (South Africa, Largest in History in 2018)
- **Contributing factors**: Environmental pathogen spread by environmental contamination, equipment, people, incoming raw ingredients (ubiquitous in nature)
- **Common in domesticated ruminates particularly** sheep, poultry, and birds.
- **Could cause sporadic and farm outbreaks in ruminants**
- **Could cause: Encephalitis, late abortion, and GI problems in ruminants**.

Growth parameters	Minimum	Optimum	Maximum
Temperature	31°F (-0.4°C)	99°F (37°C)	113°F (45°C)
pH	4.4	7.0	9.4
a_w	0.92	-	-
Other	Non-sporeformer		
Atmosphere	Facultative - grows with or without oxygen		

Sources: ICMSF 1995 and Bad Bug Book 2nd edition

Cronobacter Sakazakii

- **Recently reclassified** bacteria (2006-07), formerly known as ***Enterobacter sakazakii***
- The **Genus *Cronobacter*** was derived from the Greek term “Cronos,” a Titans of ancient mythology who swallowed each of his infants as soon as they were born (he was afraid to be replaced by his infants).
- The **species name, *sakazakii***, is named in honor of the Japanese microbiologist, Riichi Sakazaki, when the bacterium was first explained in 1980.
- Gram-negative, rod-shaped bacteria.
- Facultative anaerobic
- The growing temperature range is 6°C-45°C
- Primarily associated with **Powered Infant Formula**
- There has been several outbreaks associated with the bacterium and neonatal meningitis and death including two outbreaks in **Tennessee** (**1998 and 2001**).

APHA Compendium of Methods, Salfinger and Lou Tortorello, Fifth Edition

Cronobacter Sakazakii

Prevention (CDC & WHO guidelines)

- Breastfeed
- Practice careful hygiene
- Clean and sanitize properly
- Prepare Powered Infant Formula as recommended

Symptoms:

- poor feeding response,
- irritability,
- jaundice,
- grunting **respirations**,
- **instability of body temperature**,
- Could lead to: **seizures, brain abscess**, hydrocephalus, and developmental delay, or **death**

One of the student from class published a great article about this pathogen:

https://www.mdpi.com/2076-2607/7/3/77

APHA Compendium of Methods, Salfinger and Lou Tortorello, Fifth Edition

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