This lesson grapples with the problem of arriving at causation from correlation. In this lesson, we will re-visit Koch’s postulates, and learn how he used them to prove that anthrax bacillus \( (B.\ anthracis) \) caused an infectious disease. You will also have a chance to apply Koch’s postulates to another disease that we have already covered, cholera. Throughout this lesson, make sure to consider potential challenges to fulfilling the postulates.

**Correlation and causation: How can we determine whether a microbe causes a disease or whether it is simply associated with a disease?**

In the last few lessons, we have discussed patterns of infection. As we have seen, some diseases are passed directly from person to person through contact with bodily fluids or skin. Other infections have an environmental or animal intermediate, for example, cholera is usually transmitted when excrement of an infected person contaminates drinking water. Still other diseases, such as malaria, yellow fever, or Lyme disease, pass from person to person through a non-human vector such as a mosquito or a tick. Koch’s postulates provide us with a valuable tool for identifying the cause of infectious diseases. Once the cause is identified it can be better understood, and perhaps, prevented or better treated.

Here we will focus on the procedures that Koch used to show that anthrax is caused by the bacterium *Bacillus anthracis*, and that cholera is caused by *Vibrio cholerae*.
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Identifying the infectious agent causing anthrax: the development of Koch’s postulates

Robert Koch graduated from medical school in 1866. In the region where he started working, farmers had a serious problem with a disease epidemic in cattle. The symptoms were severe and ultimately fatal, beginning with staggering and trembling, and progressing to convulsions, bleeding from body openings, and eventually death. It was a puzzle because the disease didn’t seem to spread in an obvious way from one animal to another.

When Koch began his investigation of the cattle epidemic, anthrax bacteria had been identified, but its ability to cause disease was not yet established. His careful observations, logical thinking, and well-designed experiments allowed him to become the first scientist to establish a causal relationship between a microbe and an infectious disease. From his investigation, he came up with four postulates which he thought should be fulfilled, in order to establish causation between a microbe and an infectious disease.

1. Association — it must always be present in every case but not in healthy animals.
   When Koch took blood samples from cattle infected with anthrax and examined them under the

**Figure 1:** Koch proved that the bacilli in the blood was the cause of the cow-wasting disease. He found that only sick animals had the bacteria in the blood. Then he was able to isolate the bacteria in a pure culture. This allowed him to give bacilli to healthy animals, then he watched to see if the disease appeared. The disease did appear in infected healthy animals, and upon re-examination of the blood he found the same bacilli.

1. Match the postulate to the appropriate definition
   a. The pure microbe must cause disease in a healthy animal.
   b. It must always be present in every case but not in healthy animals.
   c. When the microbe is re-isolated from the sick animal, it must be the same as the original.
   d. It must be isolated from the sick animal into pure culture.

   _____ Association
   _____ Isolation
   _____ Causation
   _____ Re-isolation
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Microscope, he noticed that they always had large numbers of bacilli. But the cattle didn’t infect each other, so the question was: how were these bacteria involved, if at all? Were they an important causative agent, or were they there just by coincidence? Healthy animals did not have the bacilli in their blood.

2. **Isolation — it must be isolated from the sick animal into pure culture.**

He first had to separate the anthrax bacteria from all other microbes in his sample. Remember, there are ten bacteria in the body for every host cell, so simply finding the bacteria does not prove causation. Koch filtered the bacteria out of the blood of infected cattle, first grew them in broth, and later isolated the anthrax bacteria from the mixture.

3. **Causation — the pure microbe must cause the disease in a healthy animal.**

Now, that he had a pure culture of the bacterium, the next question was: will this bacterium cause disease in a healthy host? To test this, Koch injected the anthrax-containing broth into healthy animals. They got sick with the same symptoms as seen in the epidemic! This step is why isolation of a pure culture of bacteria is required. If he had used a mixture of bacteria, he would not have been able to determine which one caused the disease.

4. **Re-isolation — when the microbe is re-isolated from the sick animal it must be the same as the original.**

Koch filtered the bacteria out of the second set of sick cows, and then isolated the same bacilli again. This step provided another layer of control. For example, re-isolation helps to rule out the possibility that the original culture was contaminated with something else.

Decades later, the complete picture on the anthrax bacilli was gradually revealed. The anthrax bacteria causing the disease, *Bacillus anthracis*, survive in hostile environments as spores. The spores remain on the grass and in the soil where they are digested or inhaled by grazing animals such as sheep and cows. Once the spores enter the animal’s body, they germinate under the favorable body conditions, the bacteria replicate quickly, and produce toxins making the animal sick.

Koch proved his point by using the same principles to isolate other infectious microbes such as the bacterium that causes tuberculosis, *Mycobacterium tuberculosis*, in 1877, and the bacterium that causes cholera, *Vibrio cholerae* in 1883. In 1905, Robert Koch was awarded the Nobel Prize in Physiology and Medicine for his work on tuberculosis.

**DEFINITIONS OF TERMS**

**Germinate** — used to describe the beginning of growth of dormant life forms such as seeds and spores.

For a complete list of defined terms, see the Glossary.
Koch’s postulates revolutionized the infectious disease field and marked the beginning of the “Golden Era” of medical bacteriology. People lived for centuries without even being aware that microbes existed, let alone know they caused deadly diseases. Over a very short period of time, about 10–15 years, after his postulates were established scientists used them to prove causal relation for some of the most widespread and deadly infectious diseases such as *pneumonia*, *tetanus*, *meningitis*, and more.

**Applying Koch’s postulates to cholera**

*Vibrio cholerae* normally inhabits tropical coastal estuaries such as in Bangladesh, where it lives in close association with [phytoplankton](https://example.com). Humans can be infected when they enter this ecosystem and eat or drink contaminated food or water. High temperatures can lead to ‘blooms’ when *Vibrio* replicates rapidly, increasing the likelihood of transmission and epidemic spread. In fact, we can think of the diarrhea that *V. cholerae* causes as an evolutionary mechanism that helps the cholera bacteria spread to new hosts.

**Association**

The association postulate seems pretty straightforward: is the microbe present when there is a disease? In the picture on the left, we see a man sick with cholera. The bucket beneath his bed is filled with ‘rice-water stools’, which is a term used to describe the diarrhea of cholera victims. The rice-water stool is so-called because it is filled with white flakes resembling rice that are actually sloughed off pieces of the epithelium of the small intestine. Also, inside that bucket are billions of *V. cholerae* bacteria!

**The limitations**

There are no easy answers in science and no solution to a scientific problem is ever perfect. Koch discovered that his postulates would not be able to establish causation for all infectious diseases even if the tested microbe was indeed the cause of a disease. For example, a scientist looking for a microbial culprit for cholera would find abundant bacteria in the rice-water stool. One problem is that *V. cholerae* isn’t the ONLY bacterial species that will
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be found in the bucket. Can you think of another potential problem in establishing association? Do you think that Vibrio would be found in the stool of healthy individuals?

Isolation

In order to implicate a microbe, we need to separate it from other bacteria in a sample and grow it in pure culture. The easiest way to accomplish this is to allow it to form single bacterial colonies on solid medium with agar. Each colony is presumed to have originated from a single cell, growing as a pure culture of only one species. Most samples from the environment or a host contain mixtures of bacteria. The picture to the right shows the isolation of cholera from ‘rice-water stool.’

The limitations

Not only do you have to separate the candidate microbe from the zoo of microbes coexisting within the patient, you must also create an environment such as nutrient broth, agar gels, etc., that will support the growth of that microbe. This can be very tricky and even now scientists only know how to grow in the lab less than 1% of all bacteria!

Causation

Once a microbe has been isolated we can test its ability to cause disease by infecting a healthy subject.

The limitations

This is one of the most problematic postulates to fulfill. Many microbes only infect humans which means that you won’t have an animal model, and you can’t intentionally infect humans with cholera!

3. To isolate the V. cholera, one can
   a. strain the appropriate bacteria.
   b. do a chemical analysis to figure out the chemical components.
   c. observe the sample.
   d. manipulate the environment through nutrient broths, agar gels, etc. to support the growth of the particular bacteria.

   Figure 5: The stool sample of someone sick with cholera will be teeming with life. Culturing techniques can separate bacteria into pure culture (single colonies), allowing experiments with a select microbe.

   Figure 6: In the case of cholera the pure culture was given to healthy mice. This step is required to definitively show that a microbe causes disease, so what happens if there is no animal models?

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Another challenge is that some people are more susceptible to a certain disease than others. Remember Typhoid Mary? The typhoid microbe is an example of a bacterium that doesn’t always cause the typical symptoms of a disease. People could also have a range of susceptibilities because of immune system status, age, general health, and possibly other factors that are still unknown to us. In addition, some infections take decades cause disease making them very hard to study. For example, only 10% of people infected with *M. tuberculosis* will develop an active disease soon after they are infected. The majority, 90%, develop a latent infection (latent TB) that may progress to active disease decades later or never.

Re-isolation

Let’s assume, you have a convenient animal model for cholera like a mouse. By infecting the mouse and then re-isolating the microbe from it when it gets sick, you are attempting to confirm that the microbe that you isolated did in fact cause the disease in your test animal.

The limitations

If you infect a mouse with a bacterium that you isolated and it gets sick, but then its diarrhea does not contain the *Vibrio cholerae* bacterium that is your original candidate, it is possible that your initial identification of the candidate was mistaken or that your isolation became contaminated. In addition, microbes have short which means that they also mutate faster. A microbe may change between different hosts enough to cause difference in symptoms complicating results interpretation.

5. Re-isolating bacteria is a way of
   a. proving the correlation between bacteria and host.
   b. proving causation.
   c. confirming that the microbe that you isolated caused the disease.
   d. all of the above

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**DEFINITIONS OF TERMS**

**Latent TB** — dormant or inactive form of the disease when infected people have the bacteria in their lungs but they do not grow and divide, hence don’t cause symptoms, and can’t infect other people.

For a complete list of defined terms, see the **Glossary**.
What might prevent you from showing causation? What does it mean for Koch’s postulate of causation if only some people become sick after an infection?
<table>
<thead>
<tr>
<th>TERM</th>
<th>DEFINITION</th>
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<tbody>
<tr>
<td>Bacterial colony</td>
<td>A cluster of billions of bacterial cells that originated from one single cell, and are visible with a naked eye on the agar surface.</td>
</tr>
<tr>
<td>Causation</td>
<td>When a change in one factor results in a change in another.</td>
</tr>
<tr>
<td>Correlation</td>
<td>A connection between two factors.</td>
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<td>Germinate</td>
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<tr>
<td>Phytoplankton</td>
<td>Microscopic organisms in water environments that photosynthesize and fix carbon from the atmosphere.</td>
</tr>
<tr>
<td>Vector</td>
<td>An organism that transmits a pathogen from a reservoir to a host.</td>
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