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Vector-Borne Bacterial Agents

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Slide #1

Diseases Caused by Vector Borne Bacterial Agents

- Lyme Disease
- Rocky Mountain Spotted Fever
- Plague

Three diseases caused by pathogenic bacteria; what they have in common is that transmission of the infectious bacteria occurs via an arthropod vector.

General Objectives:
1. Understand concepts of vector, reservoir and accidental host.
2. Be aware of epidemiology that suggests a disease is transmitted by a vector.
3. Realize that the bacterium must be able to survive and grow in both the vector and in the mammalian host.

Slides #2-4

Lyme Disease

History

Lyme disease was first described in 1909 by a Swedish dermatologist who associated a bull's eye rash (erythema migrans) with tick bites.

Lyme disease was little known in the US until 1975 when several children in Lyme, CT were diagnosed with juvenile rheumatoid arthritis. This is a rare disease and it seemed odd that multiple cases popped up in the same town. Turned out to be Lyme disease! And this is how Lyme disease was named.

Early research established that Lyme disease did not spread from person to person, that most cases occur in the summer, that the disease could be treated with antibiotics, and that the distribution of cases could be correlated with distribution of the tick, *Ixodes scapularis*. This information suggests that the disease is caused by a bacterial agent that is spread by a seasonal vector such as the *Ixodes* tick.

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Willy Burgdorfer, at Rocky Mountain Laboratories in Hamilton, Montana, received some ticks and found spirochetes in them that had never been seen in *Ixodes* ticks before. He then received some blood from a Lyme disease patient on Long Island and observed the same spirochetes in the patient's blood. Patient also had antibodies to spirochetal proteins. Spirochete was named *Borrelia burgdorfei* in honor of Willy Burgdorfer.

Slides #6-8
Infectious Agent, Vector, and Reservoir:

At least three closely related species can cause Lyme disease: *Borrelia burgdorferi*, *Borrelia garinii*, and *Borellia afzelii*. Only *B. burgdoferi* is found in the US; *B. garinii* and *B. afzelii* are found in Europe and Asia.

The vector that carries *B. Burgdoferi* is the black legged tick (Ixodes). These ticks are very small and often go unnoticed. Because it is much more likely for the bacteria to be transmitted after the tick has been feeding for 2-3 days (see chart on slide), infection can often be prevented by timely removal of ticks. This is why it is important to actively check for ticks following a walk in the woods!

Slides #9-12

Ticks have a two-year life cycle and there are four stages of tick development: eggs hatch into larvae, larvae develop into nymphs, nymphs progress to adults, and female adults lay eggs. At each step in its maturation, the tick takes a blood meal. The slide photo shows a tick before and after a blood meal.

In the East and Midwest, the most common animal reservoir for the spirochete is the white-footed mouse. Deer are also important, but only because they are the principal host for the adult tick.

The nymph form of tick is the most likely to transmit the bacteria to humans. Nymphs feed during the late spring and early summer. This explains why most cases of Lyme disease occur in the summer.

Slides #13-14

On the West coast, *I. neotomae* ticks are the main vector for the bacteria and the woodrat is the reservoir. However, this species of ticks does not usually bite humans. In contrast, *I. pacificus* ticks do bite humans, but these ticks are usually not infected with Borrelia since they prefer to feed on lizards. Lizards are not susceptible to Borellia infection and so the *I. pacificus* ticks that feed on them do not pick up the bacteria and therefore are not infectious. Occasionally, *I pacificus* ticks feed on infected woodrats, become infected, and can then transmit the disease to humans. A similar situation occurs in the Southeastern United States in that the *I. scapularis* ticks found in this region also preferentially feed on lizards and thus are rarely infected.

Why aren't lizards susceptible to Borrelia infection? Lizards are resistant to B. burgdorferi due to complement-mediated killing of the bacteria in the lizard blood. We'll re-visit this issue of host range in a bit more detail a little later in the lecture.

Slides #15-16

Epidemiology
In the US, Lyme disease is found mostly in three regions:

- Northeastern and mid-Atlantic states
- Upper north-central states
- Northwestern California

Distribution of infected tick vectors mirrors the distribution of risk for contracting Lyme disease.

Slides #17-18

There has been a dramatic increase in reported Lyme disease in the last thirty years. In 1982, when Lyme disease first came under CDC surveillance, only 491 cases were reported. By 2002, 23,763 cases were reported and, for the last 10 years, 20,000-30,000 cases have been reported each year. Even these numbers are likely to be underestimates, however, as studies based on insurance claims and surveys put the number about 10-fold higher at 300,000 case per year. This increase is probably due to increased exposure to the infectious agent and also to increased awareness/diagnosis of the disease.

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Prevention:
Ticks don't fly or jump, but instead attach themselves to you or your clothing as you brush by vegetation. They then crawl up to one of their favorite spots (groin, arm pit, back of the knee, scalp) and settle in for a blood meal. Avoid this by tucking your pants into your socks and securing your shirt at the wrists.

- Avoid ticks and their natural habitats
- Wear protective clothing
- Use insect repellent
- Check for ticks after potential exposure
- Remove ticks within 24 hours

Slides #20-21

Etiologic agent

Borrelia burdorferi is a spirochete. These are long, skinny bacteria that literally look like corkscrews. They are gram-negative, contain ~7-11 flagella within the periplasmic space, and are highly motile. Having their flagella within the periplasmic space gives rise to their corkscrew motility. This type of movement is especially effective in viscous environments such as the mucosal surfaces of the body.

These bacteria are extracellular pathogens and they do not produce any known toxin. It is thought that they cause disease by their ability to migrate through tissues in the body and adhere to host cells. As we'll see, they are also quite good at avoiding the host immune system and persisting in the host.

Slide #22

Genome of *B. burdorferi* was released in 1997 (Nature, 390:580-586). Unusual in that ~40% of its DNA is carried on plasmids. Striking observation: Huge proportion of the genome is dedicated to lipoproteins, most of which are located on the bacterial surface. Highlights how important attachment is in Borrelia's lifecycle.

Slides # 23-25

Transmission from tick to mammal

Remember that tick feeding lasts for several days. As the tick feeds and blood enters the midgut, a number of environmental factors change such as temperature, pH, and nutrient availability, and these changes influence the expression of Borrelia genes encoding surface proteins. **Thus, tick feeding triggers an adaptive response in the spirochete that helps the bacterium transition from the tick to the mammalian host.**

Transition in outer surface protein expression when tick feeds: in unfed tick, OspA (Outer Surface Protein A) is high; as tick feeds, OspA production goes down and production of another outer surface protein, OspC, goes up. OspA mediates attachment of the bacterium to a tick protein (TROSPA) located on the surface of the epithelial cells lining the tick gut. These changes in outer surface proteins on the bacterial surface are thought to mediate movement of the spirochetes from the mid-gut to the salivary glands of tick and thus to mediate transmission of the bacteria between vector and mammalian host.

OspC was originally thought to mediate attachment of the spirochete to the tick salivary glands. While OspC may be involved in salivary gland attachment, it cannot be the only attachment factor since bacteria that lack *ospC* are still able to migrate from the tick midgut to the tick salivary glands. More recently, a new role for the bacterial OspC protein has been suggested based on the observation that this bacterial protein binds tightly to a tick salivary protein called Salp15. Interestingly, Salp15 has known immunosuppressive effects in the mammalian host. If the bacteria lack OspC, or if the ticks lack Salp15, the bacteria are easily cleared in the mammalian host. Only if the bacteria are producing OspC and the ticks are producing Salp15 do the bacteria survive in the mammalian host. These observations suggest the following hypothesis.
Hypothesis: Borrelia coated with tick SalP15 protein are protected from neutralizing antibodies in the bloodstream of the mammalian host. This initial immune protection could be important in a natural setting since in Lyme-endemic regions like the Northeast, most mice have pre-existing antibodies against *Borrelia*.

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In the Mammalian Host:

Once in its mammalian host, *Borrelia* uses a different set of surface molecules to bind to host cells and also to the host extracellular matrix (ECM). These interactions are thought to mediate dissemination of the bacteria to various host tissues. Consistent with the observation that *Borrelia* can infect many different locations within the body, *B. burgdorferi* can attach to a variety of cell types *in vitro*. These include lymphocytes, platelets, epithelial cells, endothelial cells, and neuroglia.

Slides # 27-30

Clinical Manifestations:
Infection starts as a localized skin infection at the site of the tick bite, but then bacteria can disseminate to other locations in the body using cutaneous, lymphatic, and blood-borne routes. See slides for symptoms and disease progression.

Slides #31-33

Immune Evasion
Most of the more serious symptoms of Lyme Disease occur after prolonged infection and reflect widespread dissemination of the bacteria. So how does *Borrelia* persist for so long in the host? By adopting the following strategies to avoid the host immune response:

1. As discussed earlier, SalP15 decoration (via OspC) during initial transition into mammalian host protects the bacteria from neutralizing antibodies.

2. *Borrelia* produces complement regulator-acquiring surface proteins (CRASPs) that bind **host** complement factor H protein. Factor H protein is a negative regulator of the complement cascade (inactivates C3b). By decorating its surface with host factor H protein, *Borrelia* is able to avoid complement-mediated killing in the blood. Like OspC, the CRASP proteins are up-regulated upon an increase in temperature and decrease in pH (which happens when tick feeds) and so are ready to protect the spirochetes when they are transmitted to the mammalian host.

Borrelia produces not just one CRASP, but a whole family of CRASP proteins. Different family members are able to interact with different factor H proteins from divergent hosts. These interactions likely play an important role in determining host range. Hypothesis: None of the CRASP proteins are capable of binding to lizard factor H protein and thus lizard complement kills Borrelia. Consequently, lizards do not support Borrelia growth and are not natural hosts.

3. Antigenic variation of surface molecules. Example: VlsE surface lipoprotein has 15 silent cassettes that can be recombined into the expressed locus.

4. Hiding in immune privileged sites

Slides #34-36

For Diagnosis and Treatment of Lyme Disease (see slides)
A few more details on diagnostic laboratory tests: CDC recommends an initial ELISA (new kit now detects Ab to conserved peptide 6 of VlsE) followed by Western blot to validate positive or equivocal results. But remember that antibody based tests are not useful during the first 1-2 weeks of infection before the body has mounted an adaptive immune response. Also, a positive antibody test does not distinguish between someone with an active infection and someone who has been exposed.

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Post Treatment Resistant Arthritis
About 10% of Lyme patients experience persistent joint inflammation for months or even years after receiving antibiotic treatment. At this point, PCR tests are usually negative for spirochetes in the joint, even though these same patients had tested positive for bacteria in their joints prior to antibiotic treatment. This suggests that the persistent joint inflammation is occurring after complete, or nearly complete, eradication of the bacteria, and has led to the hypothesis that treatment resistant arthritis is caused by an autoimmune reaction.

Key Points: Lyme Disease

- Caused by infection with a spirochete, *Borrelia burgdorfei*
- Transmitted in late spring/summer by the nymph form of the black legged tick, Ixodes
- Humans are an accidental host for the tick that normally feeds on white-footed mice
- Borrelia is able to migrate through tissues and evade immune recognition
- Illness characterized by a flu-like syndrome and a rash (Erythema migrans) and may include facial palsy, arthritis, and/or neurological symptoms
- Organism is not easily cultured or detected and so diagnosis is by immunologic evidence of infection (ELISA to detect antibody followed by Western blot to detect specific antibodies)
- Treat with antibiotics but post-infection complications problematic

Slides # 38

Rocky Mountain Spotted Fever

RMSF was first recognized in 1896 in the Snake River Valley of Idaho.
Called black measles, RMSF was a dreaded and often fatal disease.

In 1906, Howard Ricketts began studying RMSF and determined that it was spread to humans by ticks. He suspected a microbial infectious agent and but was unable to culture the bacterium. A few decades later, others succeeded in identifying a gram-negative bacillus as the infectious agent and named it *Rickettsia rickettsii* in honor of Ricketts. Rickettsia is an obligate intracellular pathogen which explains why Ricketts was unable to grow the bacteria outside the host.

Slides #39-40
The name Rocky Mountain Spotted Fever is a bit of a misnomer since most cases occur in the Southeastern and South Central states. Most cases also occur in the summer; this seasonal occurrence is consistent with a vector spread illness.

Slides # 41-43
As Ricketts had discovered, the bacteria are spread by ticks. In the Eastern U.S., Rickettsia are carried by the dog tick; while in the Western U.S., Rickettsia are carried by wood ticks. Small wild rodents serve as the animal reservoir and humans are once again accidental hosts. But unlike *Borrelia*, *Rickettsia* can be transmitted transovarially (from mother tick directly to her eggs and hence to her offspring) which allows this bacterium to be less dependent on its animal reservoir for maintenance.

Slides #44-46
Infection of the mammalian host starts when bacteria are deposited on the skin as the tick feeds. The bacteria gain entry from the host scratching the site. Symptoms appear 2-14 days after the tick bite. Disease is characterized by a sudden onset of fever and headache followed by chills, muscle aches, nausea and a “spotted” red rash. The rash starts on the periphery of the body and then spreads to the trunk.

Slide #47

Interaction with host cells:

R. rickettsii spread throughout the body via the blood stream. The bacteria attach to vascular endothelial cells and induce their own uptake. Next, the bacteria rapidly escape from the vacuole and reach the host cytosol where they replicate. Using localized polymerization of host actin at one pole of the bacteria, the bacteria move to the periphery of the cell and extend out in finger-like projections. At this point, the bacteria can either spread into adjacent endothelial cells or they can exit the host cell by lysing these finger-like projections. The resulting damage to the cell membranes of vascular cells leads to leakage of red blood cells which causes the red spots that give RMSF its name. Untreated, the bacteria destroy the blood vessels and cause death.

Slides # 48-49

Actin-based motility of Rickettsia

Actin-based motility is used by other intracellular bacteria such as *Listeria* and *Shigella*. Bacteria polymerize host actin at one end of the rod-shaped bacterial cell. This actin polymerization pushes the bacterial cell through the mammalian cell cytosol. Vaccinia virus also uses a similar mechanism to polymerize host actin.

Slide #50

RMSF Treatment and Prevention

RMSF can be difficult to diagnose since the initial symptoms are also consistent with other illnesses and because most patients don't remember having had a tick bite. In addition, while most patients develop some sort of rash, sometimes the rash does not appear until quite late in the infection. Because RMSF can be fatal within the first 8 days after the appearance of symptoms, physicians need to consider RMSF as a diagnosis even in the absence of the characteristic petechial rash. Taking into account a patient's potential exposure to ticks is important. See slide for treatment and prevention.

Key Points: Rocky Mountain Spotted Fever

- Caused by the obligate intracellular gram-negative pathogen *Rickettsia rickettsii*
- Transmitted in summer by wood or dog ticks and so most common where these ticks also bite humans
- Progressive and serious illness characterized by sudden fever and a petechial rash involving palms and soles
- Requires antibiotics effective in intracellular niche

Slides # 51

Plague

Plague is caused by the gram-negative bacterium, *Yersinia pestis*. Yersinia is capable of intracellular survival and replication in macrophages and is therefore classified as an intracellular pathogen. But Yersinia also grows extracellularly, especially late in infection (more on this later). For example, it is visible in blood smears (see slide). Here the bacteria have a bipolar appearance (i.e. they look like safety pins) and this distinctive appearance can be used as a diagnostic test.

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Main vector is the oriental rat flea, *Xenopsylla cheopis*, though about 80 species have been implicated as vectors. Wild rodents are the natural reservoir.

Slide #53-54

Yersinia colonizes the mid-gut of the flea where it grows to high numbers. The bacteria form a biofilm that clumps and adheres to the flea's proventriculus (a valve-like chamber that connects the flea's esophagus and midgut.) This blocks the ability of the flea to ingest blood and causes the flea to starve. Starving fleas feed more often, each time drawing in mammalian blood which then comes in contact with Yersinia. Because the flea can't feed normally, it regurgitates the now contaminated mammalian blood back into the bite wound and the bacteria are successfully transferred to the mammalian host. Thus, **growth of the bacteria within the flea alters the behavior of the flea and aids in transmission of the bacteria to a mammalian host**.

Slides #55-56

<u>The Disease in Humans</u>

How do people get infected? Natural hosts are wild rodents, but fleas can spread to rats, especially in cities where populations are dense. Rats can undergo epidemic from plague. When rats die, hungry fleas may turn to people. Humans are accidental hosts.

Following a flea bite, bacteria travel to lymph nodes. The lymph nodes then swell and become hemorrhagic. These infected nodes are called buboes. This occurs within a week (~2-6 days) after the initial infection and is called bubonic plague. In addition to buboes, symptoms of bubonic plague include high fever, chills, headache, and extreme exhaustion.

Slides #57-58

If not treated immediately with antibiotics, the bacteria can spread into the bloodstream and produce a systemic infection. This form of plague is called septicemic plague and is very dangerous and often fatal. Hemorrhaging of blood vessels in the extremities (fingers, toes, nose) causes these areas to be deprived of oxygen; the resulting necrosis turns the skin black and is what gives the disease its nickname "Black Death". In addition, once the bacteria get into the bloodstream, they sometimes reach the lungs, resulting in pneumonic plague. At this point, the bacteria do not need fleas to spread them; they can be passed directly from human to human via airborne transmission. **Pneumonic plague results in dangerous epidemic conditions**.

Slide #59

Plague History

There have been three major pandemics of plague in history. First pandemic hit Constantinople in 542 A.D and then spread into Europe. People died at a rate of about 10,000 per day and close to half the population was lost. Second pandemic started in China and spread along the Silk Road to Europe in the middle of the 14th century and was the worse disaster in human history. The death toll was staggering. 25 million people in Europe died (about half the population) in five years. In the Islamic world, about a third to half the population died. And in China, about half the population died. The third pandemic arose in China in 1894 and spread throughout the world via modern transportation. Ships carried rats, fleas, and plague and spread the disease far and wide. The disease arrived in the U.S. around the start of the 20^{th} century, most likely via Chinese laborers arriving in San Francisco.

Slides #60-61

Pathogenesis:

It was during this third pandemic that Alexandre Yersin discovered the plague bacillus, *Yersinia pestis*. Yersinia is a gram-negative, rod-shaped bacterium. It is classified as an (facultative) intracellular pathogen due to its ability to survive and grow in macrophages. This ability may have been initially selected for as a way to avoid intracellular killing by amoebae in the environment. The role of intracellular growth in a mammalian host is not entirely clear. Because Yersinia is readily killed by neutrophils when grown at 25-28^{0}C (the temperature of a flea), it may use its ability to survive in macrophages as a way to hide early in mammalian infection until it can successfully turn on its virulence factors. When the bacterium transitions from a flea to a mammalian host, the increase in temperature triggers a change in gene expression that results in production of Yersinia's virulence factors. These virulence factors act in concert to prevent phagocytosis later in infection. As a result, Yersinia is usually observed growing extracellularly in a mammalian host, especially late in infection. These virulence factors include production of capsule and a Type Three secretion system (TTSS). Using its TTSS, Yersina injects bacterial effector molecules directly into the cytosol of the host cell. These bacterial effector proteins prevent phagocytosis by perturbing the host cell actin cytoskeleton. In addition, other bacterial effector molecules perturb host cell signaling cascades and slow the inflammatory response of the host. If left untreated, the bacteria usually get ahead of the host immune system and overwhelm it.

Slides #62-64

Present Day Plague:

With the advent of modern sanitation in cities, plague is no longer the problem it once was. However, outbreaks still occur. An example happened in India about 20 years ago with ~700 confirmed cases (~5,000 suspected cases) and 53 deaths. More recently, plague has become an issue in Africa. Over the last ten years, almost all reported plague cases have occurred in Africa, primarily in Congo, Uganda, and Madagascar. For example, in the last decade, Congo has had over 10,000 reported cases of plague and Madagascar has had over 7,000 cases. Annually, there are about 2,000 reported cases of plague worldwide.

In the United States, plague is relatively rare with only about 5-10 cases reported per year. Most cases occur in the rural and semi-rural Southwest because that is where the most important animal reservoirs for plague (ground squirrels and prairie dogs) are found. People get infected from flea bites or from handling infected animals, their tissues, or their pelts.

Slides #65-66

Diagnosis, treatment and prevention

Traditionally, plague has been confirmed by microscopic examination of Gram or Giemsa stained smears from patient's blood and/or buboes. If necessary, a fluorescent antibody test for a *Y. pestis*-specific envelope antigen can also be performed. Just this year, however, the CDC has developed a new diagnostic tool and is trialing it in Uganda. This is a dipstick that can detect the presence of plague bacteria in a patient's blood without the need for a microscope.

If caught early enough, most plague cases can be successfully treated with antibiotics. Streptomycin is the antibiotic of choice here. If pneumonic plague is suspected, then isolation of patients is important to prevent spread of the disease. Control of rodent populations, especially in urban settings, and use of insecticides are useful in preventing plague.

Key Points: Plague

- Caused by the Gram-negative intracellular pathogen, *Yersinia pestis*
- Transmitted by fleas that normally feed on wild rodents, but can be transmitted human to human via airborne transmission (pneumonic plague)
- Progressive and severe disease characterized by fever and painful, infected lymph nodes (buboes)
- Important animal reservoirs in the US are prairie dogs and ground squirrels