Welcome back to lecture 9, the foodborne pathogens. In this lecture we are going to talk about some things that you probably haven’t thought could be in your food. Including worms and protozoa. In the picture at top right you can see worms attached to the stomach wall. On the other side you can see a very common protozoan known as *Giardia*.

The objectives for this lecture are as follows; we are going to list and describe the protozoal pathogens found in food. We will list and describe the helminthic pathogens found in food. Please remember that helminthic means worms! We are going to describe the unique prion pathogens and the role of food in their transmission.

To start with we will talk about the foodborne protozoa. The protozoa include *Giardia, Cryptosporidium, Toxoplasma gondii*, and *Cyclospora cayetanensis*. As a note, there are plenty of other protozoa that can be found in foods. However, in the interest of time, we are only discussing those found in the US food supply. I want to remind students that I will ask questions regarding what type of organism was responsible for a disease. For example, on a test I might ask, *Giardia lamblia* is which of the following? A protozoan, a worm or a bacterium? So please be sure to pay attention to which type of organism is being discussed.

I want to draw your attention to the protozoan life cycle. Many of us are very familiar with bacterial and viral life cycles but may not have seen a protozoan life cycle. The infectious stage of a protozoan is usually the cyst. This is the round thing in the figure that looks like it has four eyes. The cyst is what is ingested. However, once you eat it, it can become a trophozoite. Trophozoites may pass in the stool but they do not survive in the environment because they are not tough. The cyst on the other hand is very tough and act similar to a bacterial spore. It is also important to note that the trophozoite is motile, it swims!
Giardiasis caused by *Giardia lamblia* is spread by ingestion of water contaminated with cysts. It is the most frequently diagnosed intestinal parasite in the United States with 2.5 million cases diagnosed every single year. The symptoms include cramps, nausea, and diarrhea. Again we see very non-specific symptoms. It has a very low infectious dose which is important because you do not have to eat or drink much to get sick. They are also chlorine resistant so you should be thinking about how would we kill this? You may be aware that water entering a water treatment plant such as the one here in Tampa is highly contaminated. There are a number of different ways in which that water is purified before it is piped out to your house. Since this (and other parasites) are not killed by chlorine, we use filtration in the water treatment process to remove them. The parasites are quite large and easily removed by filtration. The treatment for this disease is metronidazole which is sold under the brand name Flagyl. Please take a look at the trophozoites in the stool sample on the right. They are very big and if you look at them in the microscope they look as if they are looking right back at you!

*Giardia lamblia* outbreaks are most commonly associated with contaminated water sources such as rivers, lakes, and swimming pools. Foods washed with these contaminated waters are also a source of outbreaks. On the right is a case report of *Giardia* in the corporate office which is unusual. Most outbreaks occur amongst people out in the woods, drinking from lakes, people swimming in common areas, etc. *Giardia* is spread by the fecal-oral route and therefore spread in daycares occurs.

Another important disease is cryptosporidiosis spread by a number of *Cryptosporidium* species. The disease occurs due to ingestion of oocysts from contaminated water. We will not worry too much about the life cycle of this pathogen but think of an oocyst as a small cyst. Foods washed with contaminated water can also be infected. Symptoms include watery diarrhea, weakness, fever, nausea, and abdominal pain. Just like *Giardia*, *Cryptosporidium* has a low infectious dose and is chlorine resistant. Again water sources must be filtered to remove the organism. The treatment for this is rehydration and Bactrim.
In 1993 a massive *Cryptosporidium* outbreak occurred in Milwaukee due to filtration failure at a water treatment plant. Remember this organism is resistant to chlorine and you must use filtration to remove it from the water. It is estimated that the number of people infected ranged from 400,000 – 600,000 and unfortunately 104 people lost their lives. Most of those who died were immunocompromised. It is interesting to note that this outbreak was discovered because stores were running out of drugs used to treat diarrhea. It was thought to have occurred due to sewage runoff. Originally it was thought to be caused by cows that were upstream of the treatment plant but DNA testing later showed the cows were framed! Not surprisingly, when you look at the epidemiology curve (on right) we see something very characteristic of a common source outbreak.

We change gears here to talk about toxoplasmosis caused by *Toxoplasma gondii*. If you are familiar with this pathogen the first thing that popped into your mind was cats. That is because cats are the primary reservoir host. It is important to note that cats may not have any symptoms of the disease. You get this from ingestion of cysts and/or oocysts from soil, litter, raw meat (including beef and pork). Immunocompromised persons are at high risk of infection and death. Diagnosis of this disease is difficult because the cysts circulate in the body. They can end up in the brain and eyes and this disease can be fatal. Unfortunately, for a fetus is usually fatal. This is the reason why pregnant women should NEVER change cat litter.

If you look at the life cycle here you will note that if a pregnant woman becomes infected, what is known as tachyzoites can infect the fetus via the bloodstream. Unfortunately this often results in miscarriage or severe development problems. Cats do not have symptoms and therefore pregnant women should NEVER change cat litter.
Toxoplasmosis – *Toxoplasma gondii*

- **4th** leading cause of foodborne hospitalization (8% overall).
- **2nd** leading cause of foodborne death (24% overall).
- Pathogen-food combinations most commonly responsible for illness are pork and beef.
- Recent studies suggest more than 50% of *T. gondii* cases are due to undercooked pork.
- Prevent by proper cooking.

Note this pork tenderloin is pink in the middle so it cannot have reached the recommended cooking temperature.

I wanted to be sure you know the importance of cats in the spread of *Toxoplasma* but as this course is food safety, we want to return to food on this slide. *Toxoplasma* is the **4th** leading cause of foodborne hospitalization. It is the **2nd** leading cause of foodborne death. The pathogen-food combinations most commonly responsible for illness are pork and beef. What this means is when you diagnose *Toxoplasma*, the food most likely responsible is undercooked pork or beef. Recent studies suggest that more than 50% of *T. gondii* cases are due to undercooked pork. On the right you can see a picture of a pork tenderloin. Very commonly they are lightly cooked on the outside and have not reached the proper temperature on the inside. It is pink in the middle so it is possible that it has not reached the proper temperature (note: in some cases pink meat may have reached the proper temperature so be sure to default to using a thermometer). Obviously, toxoplasmosis can be prevented by proper cooking.

Toxoplasma has been associated with horse meat consumption (considered a delicacy) in Europe. The US has approved a plant to process horse meat but the plant has never (as of late 2014) opened due to strong opposition.

You may recall this story as it caused quite a stir when horse meat was found in IKEA meatballs. If you are familiar with the IKEA stores, you know they have restaurants inside and one of their biggest delicacies is the meatballs. Unfortunately, testing revealed that some meatballs contained horse meat. *Toxoplasma* has been associated with horse meat consumption. Horse meat is considered a delicacy in Europe. There has been talk about opening a horse meat slaughter plant in the US. However, there is very strong opposition here. The US has approved the plant but there has been so much opposition that it has not yet opened (still true in late 2015). If we decide to put horse meat in the US food supply, we will have to be concerned with *Toxoplasma*.

*Cyclospora cayetanensis*

- Causes watery and explosive diarrhea, loss of appetite, and bloating.
- Foods implicated include imported strawberries, raspberries, and raw vegetables.
- Good sanitation is prevention.
- There is no treatment.

*Cyclospora* can be considered an emerging pathogen as it has recently become a bigger problem in the US. The life cycle is shown on the right and note they included raspberries and basil in the picture as both have been associated with outbreaks. It causes watery and explosive diarrhea (remember you can tell a lot about the type of poop that occurs), loss of appetite, and bloating. Foods implicated include imported strawberries, raspberries, and raw vegetables. [Note added: cilantro has been implicated in outbreaks in 2014 and 2015]. Good sanitation is prevention as this pathogen is spread via the fecal-oral route. Important to wash foods thoroughly. There is no treatment [Note added: Bactrim has recently shown some promise and is currently recommended, however, there is no recommended alternative for those allergic to Bactrim (a sulfa-drug)].
In 2013, a large outbreak of cyclosporiasis occurred in the United States. There were 631 people who were infected. The source of the outbreak was cilantro from a fresh salad mix sold by Taylor Farms of Mexico. Please note that Taylor Farms is a very large company. There is a Taylor Farms of Florida but they were not implicated in this outbreak. There is also a Taylor Farms of California which was implicated in an unrelated outbreak. Cyclospora often is imported. It occurs in tropical and subtropical regions and outbreaks in the US are pretty much always caused by imported foods. Again recall that global food trade can introduce pathogens. On the right you can see which states were affected by the outbreak.

This brings us to the really gross part, the worms! If you look on the right here you see a guy laying out a tapeworm. Some tapeworms can get extremely long. The story is about a woman who tried the tapeworm diet. Unfortunately, she tried to buy a tapeworm off of the internet, swallow it, and lose weight. This was a very common diet and this has been banned in the United States. The foodborne helminths include *Taenia saginata*, *Taenia solium*, and *Trichinella spiralis*. Please note that there are lots of other worms out there but these are the ones most commonly diagnosed in the US. These are often the result of travel or illegal importation of foods. One thing to note that some worms are also found in fish that are not pathogens and therefore not covered here.
It is really the pork tapeworm that is much more dangerous. This is *Taenia solium*. It is transmitted by undercooked pork containing encysted larvae. Symptoms and treatment are the same as *Taenia saginata* with the exception of cysticercosis. Cysticercosis is a serious disease that occurs when eggs instead of larvae are ingested. If you eat the pork tapeworm eggs, they can travel to muscles, the eye, and the brain causing a fatal disease known as neurocysticercosis. If you look at right you can see a picture of a brain in a person with this disease. As you can imagine, this is often confused for other diseases such as prion disease and cancer. However, if the skull is opened for surgery, as has happened in the past, sometimes larvae are present in the brain.

This brings us to trichinellosis cause by *Trichinella spiralis*. You can see the larvae curled up here on the right. It is a roundworm transmitted by ingestion of raw or undercooked pork containing the *Trichinella* cyst. If you get these larvae, they migrate to muscles included the eye, the tongue, and the diaphragm. You will recall in an earlier lecture I mentioned this could only be diagnosed by muscle biopsy. Remember that picture in which they took the little device and punched out a piece of tissue from the person’s lip. Treatment for this is mebendazole but I would like to you to consider that moving pathogens once killed remain in place where they continue to be attacked by the immune system. Therefore, they can still cause problems. Pork is the most commonly implicated food group and infection can be prevented by properly cooking pork. Recently, the number of cases from pork is decreasing while the number of cases from ingestion of wild game is increasing. It is important to note that some foods that are uncommonly eaten known as wild game and discussed later can contain pathogens. You will note with the last three pathogens, beef tapeworm, pork tapeworm, and pork worm, I have not given you examples of outbreaks. This is because they are generally imported and rarely involve more than 1 or 2 people at a time.

As I mentioned, *Trichinella* can be a problem for hunters and the CDC does have a webpage that warns that black bears and wild hogs can harbor *Trichinella*. You should take care to fully cook the meat from these animals. If you hunt and field dress them, be careful not to introduce one of these pathogens [note added: from cuts, etc].
Students often ask at this point why we have not talked about toxins. The reason is that they will be covered in another lecture. There are number of toxins that are produced by fungi, algae, and bacteria that also lead to foodborne disease. These toxins will be presented in Lecture 11 – Food Toxicology. I also want you to note this quote at the bottom. This is from Neil deGrasse Tyson and he says that the likelihood that a person used the word "toxin" correlates strongly with how much Chemistry that person does *not* know. This is important because people often like to say that our food is “full of toxins”. Scientifically speaking that is not correct. Your instructor believes you should always speak out in defense of science!

This brings us to a totally different type of pathogen, the prions. Prions are very unusual in that they are proteins. We tend to think of infectious things as being viruses and bacteria and not proteins so these are very unusual. Prions are components of the neuronal cell membrane. I am showing you a picture on the right of the structure of the neuron. You don’t need to memorize this picture but it may be helpful if you are taking anatomy and physiology or to remind you if you took an intro biology course. Abnormally folded forms of the prion are infectious and are the cause of disease. The word prion means protein and infectious.

How do prions actually cause disease? Normal prion protein (PrP\text{C}) is converted by abnormal folding into (PrP\text{SC}). PrP\text{SC} forms into an infectious particle, named a “prion” that can transmit disease. In other words, an abnormal prion protein teaches a normal protein to be abnormal. Accumulation of PrP\text{SC} in the brain causes neurodegeneration. Those of you interested in neurodegenerative diseases should study prions. Abnormally folded proteins have opened all new avenues of research in neurodegenerative diseases.

Because this is complicated, this slide just states the same thing in a different way. Why prions cause disease. Prion protein can exist in two folded conformations. Mutated prion protein can cause normal protein to mutate. In other words, when mutated prion protein appears, it teaches normal protein to mutate. Many have suggested that prions behave similar to viruses and should be categorized as viruses. However, one could argue that they actually behave like some normal proteins. As I said earlier, in neurodegenerative diseases, we have found normal proteins that do the same thing.
Transmissible spongiform encephalopathies (TSEs)

- Some diseases caused by prions are known as transmissible spongiform encephalopathies due to the appearance of the brain on autopsy (large vacuoles in the cortex and cerebellum).
- All are fatal neurodegenerative disorders of humans and animals associated with accumulation of abnormal forms of prion protein in nerve cells.
- Common symptoms include loss of motor control, dementia, paralysis, wasting, and death.

Quite literally, prions cause holes to appear in the brain.

I wanted to show you the prion list of diseases. We will not cover all of these in this lecture as we want to concentrate on those that are foodborne. There are a large number of these diseases. In humans there is the Creutzfeld-Jakob disease with three different types. You can see the other diseases here and we will briefly talk about kuru. In the animals there are a number of diseases that can cause humans problems because we eat animals. There is scrapie, bovine spongiform encephalopathy, chronic wasting disease, transmissible mink encephalopathy. If you eat any of the animals susceptible to these diseases, you could be in trouble.

Sometimes diseases jump species barriers which is likely what happened with mad cow disease, the disease we will concentrate on the most. You should know that this originated as scrapie. The disease occurs in goats and the animals undergo behavioral changes including tremors and pruritus (the name originated from the animals attempting to scrape the fleece off, and incoordination. Probably crossed the species barrier into cows. In other words, this disease probably resulted in mad cow disease (BSE).
Mad cow disease is known as bovine spongiform encephalopathy or BSE. It was first reported in the UK in 1986. Over 180,000 cases confirmed. 1-3 million cattle probably infected. It appears that sheep that had scrapie were ground up into feed that was fed to cows. This allowed the organism to jump the species barrier causing mad cow disease.

Continuing this theme, the infected cows were then eaten by humans. This probably resulted in the human disease known as variant CJD due to ingestion of contaminated beef. We know this because of the space/time correlation, later biochemical evidence, and finally animal models of disease. The theory is that humans ingested beef containing BSE prions which “taught” human prions to misfold leading to vCJD (variant Creutzfeld-Jacob Disease). Laboratory experiments have confirmed that this can occur.

If you want to look at the timeline to keep things straight. It first started out in sheep, then it jumped into cattle, then it jumped into people. In humans the disease develops over a long time. It causes degeneration to vital parts of the brain, causes dementia, weakens muscles, and causes imbalance.

The disease known as CJD is actually genetic. Therefore this disease which looks very similar is called acquired or variant CJD. The prion present in patients with vCJD is indistinguishable from BSE which you will remember is bovine spongiform encephalopathy. Early in illness patients experience psychiatric symptoms usually depression, occasionally schizophrenia-like psychosis. This is unfortunate as many of the original cases were hospitalized with suspected psychological disorders. Other symptoms include unusual sensory symptoms, unsteadiness, difficulty walking, and involuntary movements. Immobility and muteness occur by time of death.
You may be asking yourself why I keep showing you cartoons and giant microbes. The reason is that the pictures are quite sad and the animal pictures look quite cruel. Some of the mad cow disease pictures have been used by animal rights activist in an attempt to show mistreatment of animals when these are really pictures of diseased cows. [note added: in other words, it is hard to determine anymore which pictures are really mad cow disease]. All prion diseases are fatal. Patients are provided with symptomatic treatments. Animals are destroyed. As you can imagine this was very destructive to the beef supply in the UK as no one would buy their products for many years. Even to this day UK citizens are prohibited from donating blood in the United States out of fears of mad cow disease. It is difficult to deliver drugs through the blood-brain barrier which is one of the reasons this disease is so hard to treat. When you learn more pharmacology if you progress in the health sciences, you will learn that this is a huge problem with a large number of drugs. Many substances do not cross the blood-brain barrier. Take a minute to consider which substances do cross the blood-brain barrier.

Looking at disease distribution, the exact number of cases is unknown but new diagnostic tests are available. There is a immunohistochemical test that uses tonsil tissue to determine if someone has the disease. When you look at the data on right you will note that most persons have already died. This is a long term disease and symptoms may not occur until a long time post-exposure, but it is 100% fatal.

If we look at the impact of prion disease we note that it has great economic impact on the beef industry. If we had beef infected with prion diseases in the United States, it would do some amazing economic damage to our exports. There were thousands of animals destroyed in the original outbreak and they still have to be destroyed anytime this is identified. There were 165 cases (158 deaths) due to vCJD in UK. There were three US cases (vCJD) identified. UK residents/visitors banned from blood donation. Consumer hysteria regarding safety of beef products would be a huge issue if this pathogen were identified in US beef products.
I wanted to tell you real quickly about kuru because this is an interesting story. You could argue whether or not this is foodborne but you do get it by eating. Over 3,000 fatalities are known. Disease transmitted by cannibalistic mourning practices of affected individuals. The kuru prion is transmitted by ingesting the brain of the person who died. You may be asking yourself who in the world would eat a dead person’s brain? But it is believed that ingesting the brain will result in gaining the dead person’s knowledge. This was found in Papua New Guinea and there are different tribes there that practice this cannibalism. Numerous symptoms including progressive fatal ataxia or loss of muscle coordination. Unfortunately, it is fatal and the person dies, so immediately, someone will ingest the brain. Currently Papua New Guinea is the only country known to practice cannibalism and the practice has been banned by local governments. What is interesting is that kuru was rarely reported in Papua New Guinea to begin with but there have been no new cases in a number of years. Some people believe the disease has been eradicated. There are very few diseases which have been eradicated but this may be one of them.

How do we prevent prion diseases? Prions are highly resistant to most physical and chemical methods used to inactivate pathogens. Autoclaving and incineration are effective but you must keep in mind what can and cannot go into the autoclave. Carcasses of infected animals must be incinerated. Infected feed animals must be destroyed. Milk from infected cows must be destroyed. Human and animal tissues, blood, etc., must be tested prior to transplantation. This is important because we know prions have been transmitted by corneal transplantation. Introduction of transgenic cows lacking normal cellular prion protein. This is an unusual idea that we are going to cover to later lectures. There is in a laboratory a GMO cow that cannot get mad cow disease. This is unusual in that there are very few GMO animals but someone in a lab was able to create a cow that cannot get prions. This could potentially be a beef industry saving animal. Surveillance in animal population must continue. Anytime we detect a prion we must destroy the animals.

Hopefully you have enjoyed learning about some unusual pathogens in this lecture. Protozoal infections are largely spread in fecally-contaminated foods and water. Helminthic infections spread by food are rare in the United States but can have deadly consequences. The prions are a unique category of infectious agents which cause fatal disease and are difficult to prevent.

[Note added: instructor misspoke here on the audio, prions are difficult to prevent, they are impossible to treat at this time].