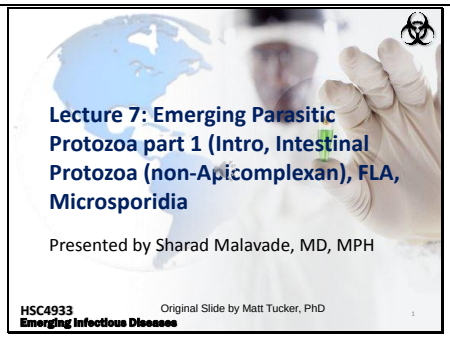
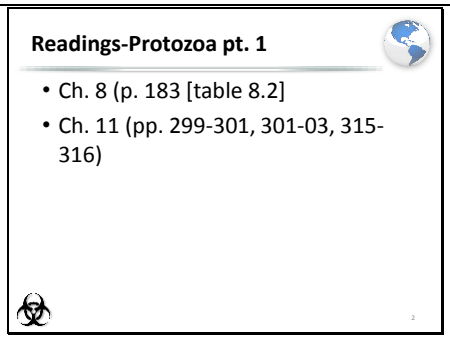
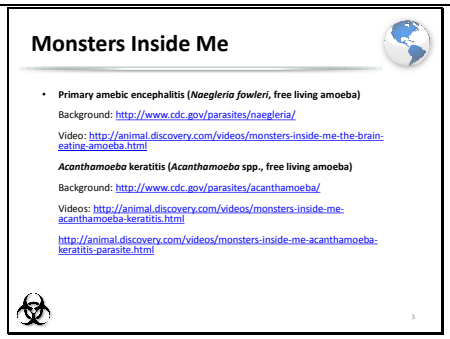
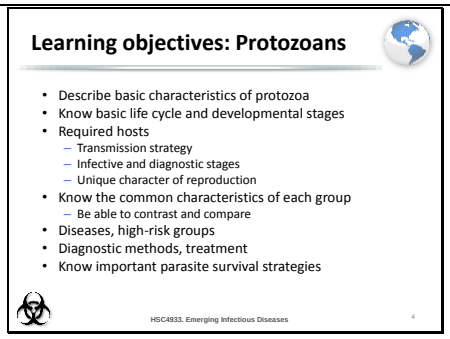
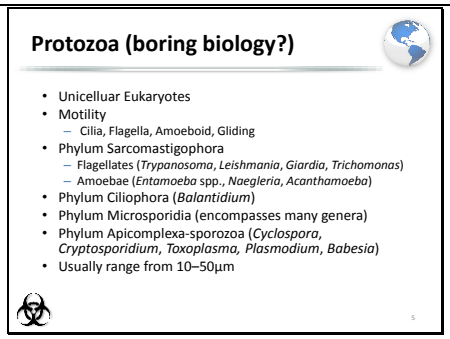
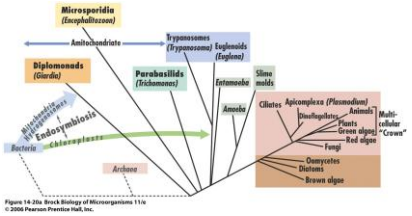
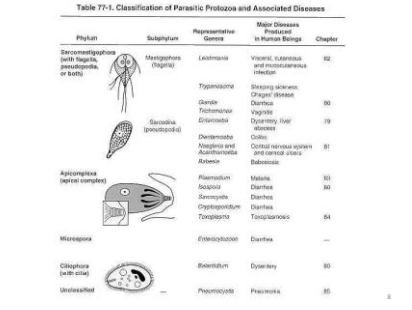
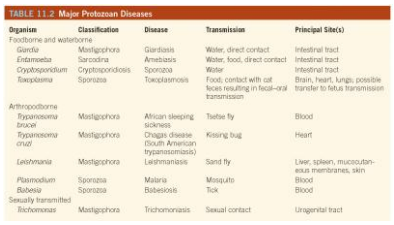
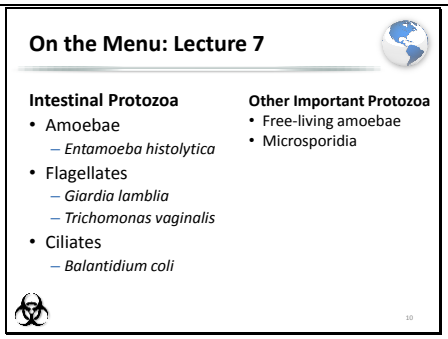
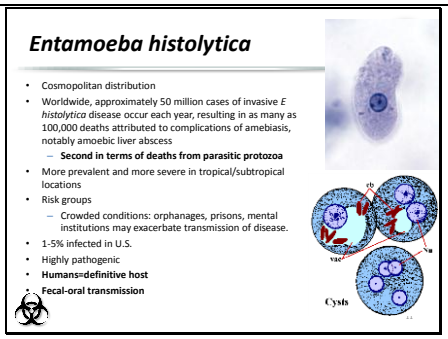
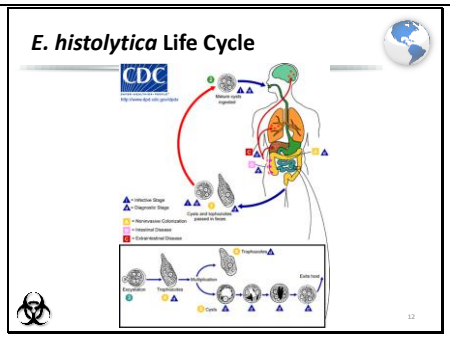
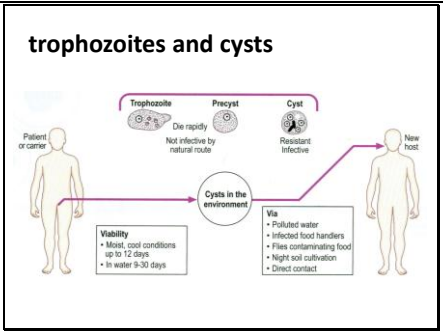
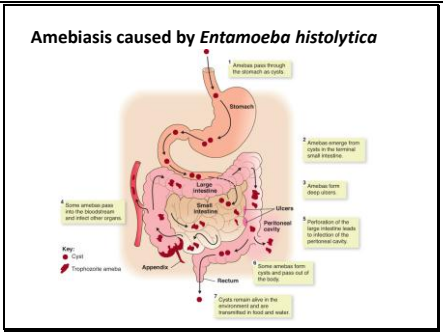
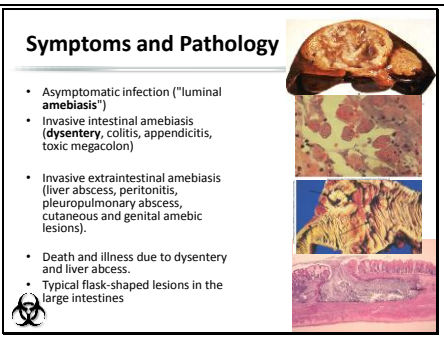
Slide 1		This lecture begins with the protozoan parasites. This the first part that covers the introduction to protozoan parasites, intestinal non-apicomplexan parasites, free living amoeba and micosporidia.
Slide 2		These are the readings for this lecture.
Slide 3		These are some interesting websites that you can check out for some background information and interesting instances when parasites have infected people.

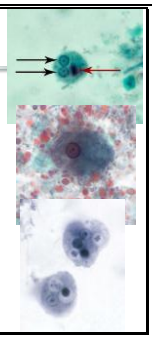
Slide 4		These are the learning objectives for this lecture.
Slide 5		This is a brief overview of the different protozoal parasite phylology just to understand where they are placed taxonomically. The protozoa are single celled and have a distinct nucleus. They move around by means of structures like cilia and flagella or use amoeboid or gliding movements. The Phylum Sarcomastigophora has the flagellates (Trypanosoma, Leishmania, Giardia and Trichomona) and the Amoebae (Entamoeba spp., Naegleria, Acanthamoeba) Phylum Ciliophora (Balantidium) Phylum Microsporidia (encompasses many genera) Phylum Apicomplexa-sporozoa (*Cyclospora*, *Cryptosporidium*, *Toxoplasma, Plasmodium*, *Babesia*) These parasites usually range from 10–50µm in size.

Slide 6	**Taxonomic Overview**  Figure 14-20a Brock Biology of Microorganisms 11/e © 2006 Pearson Prentice Hall, Inc.	This figures just shows the toxonomic relationships. This wont be asked on the exam.
Slide 7	**Parasitic Protozoa** • Infect a variety of hosts • Multiplication within hosts, enabling huge numbers in short periods • 50,000 species of protozoa, of which a fifth are parasitic • Life cycles – Usually less complex than helminths – Many examples of direct and indirect – Cyst form offers protection against harsh conditions, allowing to survive extreme temperatures or harmful chemicals or lack of food, water, or oxygen	The parasitic protozoa infect a variety of hosts that includes humans, animals and birds. They multiply within their hosts in huge numbers within very short periods. They have evolved to utilise the short favorable conditions of their living environment to their maximum advantage. There are about 50,000 species of protozoa of which about a fifth are parasitic. Their life cycles are usually less complex than those of helminths. In hostile environmental conditions, the protozoal parasites can form cysts that help them survive extremes of temperature, moisture, pH or harmful chemical and lack of food, water or oxygen.
Slide 8		Here is a table that shows the parasitic protozoa that we will be covering and the diseases they cause.

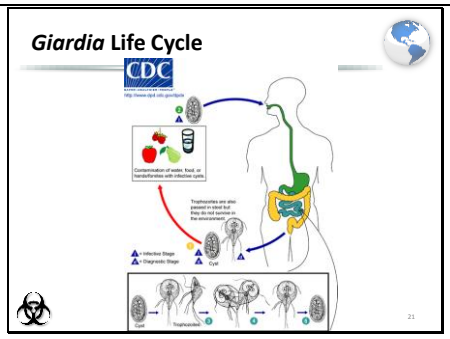
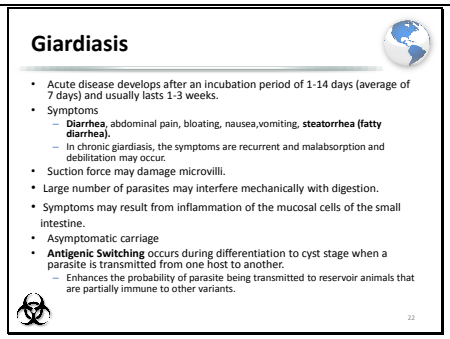
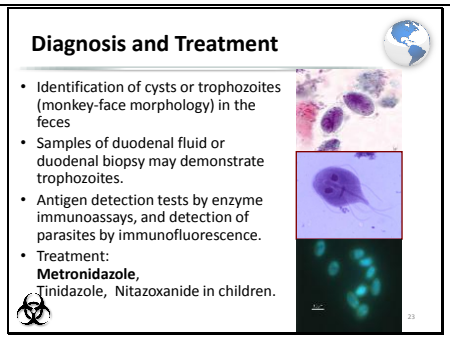
Slide 9	 TABLE 11.2 Major Protozoan Diseases Organism / Classification / Disease / Transmission / Principal Site(s) Foodborne and waterborne *Giardia* — Mastigophora — Giardiasis — Water, direct contact — Intestinal tract *Entamoeba* — Sarcodina — Amebiasis — Water, food, direct contact — Intestinal tract *Cryptosporidium* — Cryptosporidiosis — Sporozoa — Water — Intestinal tract *Toxoplasma* — Sporozoa — Toxoplasmosis — Food; contact with cat feces resulting in fecal–oral transmission — Brain, heart, lungs; possible transfer to fetus transmission Arthropodborne *Trypanosoma brucei* — Mastigophora — African sleeping sickness — Tsetse fly — Blood *Trypanosoma cruzi* — Mastigophora — Chagas disease (South American trypanosomiasis) — Kissing bug — Heart *Leishmania* — Mastigophora — Leishmaniasis — Sand fly — Liver, spleen, mucocutaneous membranes, skin *Plasmodium* — Sporozoa — Malaria — Mosquito — Blood *Babesia* — Sporozoa — Babesiosis — Tick — Blood Sexually transmitted *Trichomonas* — Mastigophora — Trichomoniasis — Sexual contact — Urogenital tract Another nice review slide	This is another nice review slide taken from the previous edition of the textbook. It shows the organisms that we will be covering in the course. It show classification of the organism, the disease it causes =, transmission routes and principle sites of infestation/infection.
Slide 10	 **On the Menu: Lecture 7** **Intestinal Protozoa** • Amoebae – *Entamoeba histolytica* • Flagellates – *Giardia lamblia* – *Trichomonas vaginalis* • Ciliates – *Balantidium coli* **Other Important Protozoa** • Free-living amoebae • Microsporidia	So for this lecture we will talk about the intestinal protozoal parasites, Entamoeba, flagellates Giardia and Trichomonas, the cliiates, Balantidium and the free living amoebae, Acanthamoeba and Naegleria.
Slide 11	 ***Entamoeba histolytica*** • Cosmopolitan distribution • Worldwide, approximately 50 million cases of invasive *E histolytica* disease occur each year, resulting in as many as 100,000 deaths attributed to complications of amebiasis, notably amoebic liver abscess – **Second in terms of deaths from parasitic protozoa** • More prevalent and more severe in tropical/subtropical locations • Risk groups – Crowded conditions: orphanages, prisons, mental institutions may exacerbate transmission of disease. • 1-5% infected in U.S. • Highly pathogenic • **Humans=definitive host** • **Fecal-oral transmission** Cysts	Entamoeba histolytica is the significant Entamoeba from a human health perspectivie. It is highly pathogenic and has a worldwide distribution. Annually it infects approximately 50 million cases. And results in about a 100,000 deaths from complications primarily liver abscess. It is more prominent in the tropical and subtropical climes. Situations that result in crowding of human habitation such as prison, mental institutions are known to have increased transmission rates of this parasite. It has also been shown to have high transmission in men who have sex with men. Humans are the only definitive host and the transmission is fecal-oral. In U.S., more prevalent in rural areas- SE and SW parts of country in lower, socioeconomic groups

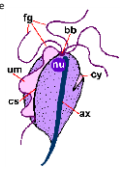
Slide 12		The life cycle of E. histolytica is pretty simple. The infected persons pass out either the trophozoites or cysts, in the environment, the trophozoites usually transform into cysts. They can contaminate food or water especially if the carrier or the infected person does not wash hands carefully with soap and water after ablutions. Another person consuming the contaminated food or water ingests the cysts. The cysts excyst in the intestine forming trophozoites and then pass the cysts or trophozoites out in feces and the cycle is completed. Important things to know about this • Trophozoites: This is the stage of the parasite that feeds. So it is also the stage that will cause the host tissue to dissolve (lyse=breakdown) and thus it will consequently invade the host tissues. So this stage is also called the invasive stage. • Cyst: It is the stage that is parasite in survival mode and can withstand hostile environmental conditions. Under favorable conditions, the parasite excysts (ex=out) of the cysts to assume the trophozoite stage. • Intestinal amoebiasis: When the infection of the parasite is limited to the intestinal tissues. It can have both cysts as well as the trophozoites. • Extra-Intestinal amoebiasis: When the infection of the parasite is in non-intestinal tissues. The way this happens is sometimes the trophozoites cause breakdown of a blood vessel during tissue invasion. Usually it's a tributary of the portal venous system that takes the nutrient rich blood from the intestines to the liver. Once in the liver, the trophozoites lodge in the hepatic sinusoids and lyse liver tissue and incite inflammation- eventually forming amoebic hepatic abscess. They can also be distributed to other organs like the brain and lungs.

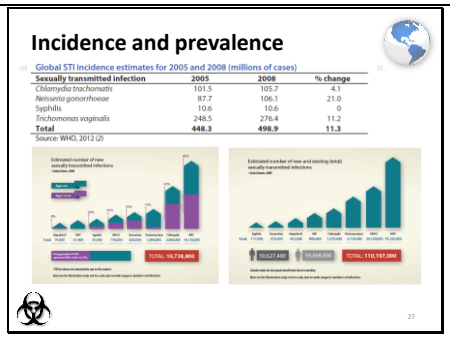
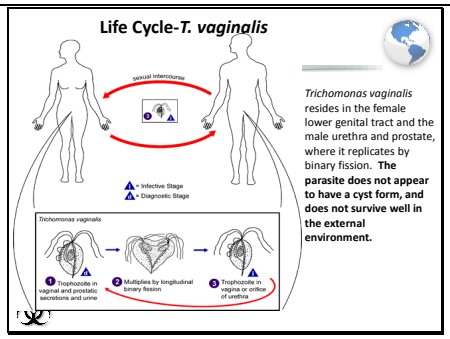
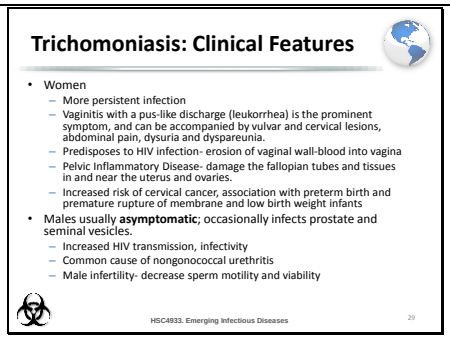
Slide 13		This graphic shows the important characteristics of trophozoites and cysts. Cysts are the infective form and trophozoites are the invasive forms. Trophozoites are fragile and by themselves would not survive in the outside environment. This schema can be generalised to most of the intestinal protozoal parasites.
Slide 14		This diagram shows the cyst forms causing the infection and the trophozoites causing the invasive disease. The red round organisms are the cysts whilst the irregular shape red figures represent the trophozoites.
Slide 15		The infection due to E. histolytica can be divided in to three types. • Asymptomatic luminal infections. In this the organism survives in the lumen without invasion of the intestinal tissues. • Invasive Intestinal infections. This is characterised by abdominal pain, dysentery and be due to appendicitis, colitis and sometimes even toxic megacolon. Dysentery=severe <u>diarrhea</u> containing <u>mucus</u> and/or <u>blood</u> in the feces. Toxic megacolon results when the colon muscles lose their tone due to either the toxic effects of the infection or the effect of the parasite on the innervation of the colon. Another parasite that does cause toxic megacolon is the parasite of Chagas disease *Trypanosoma cruzi* in Latin America. • Extraintestinal infections can be in the liver, lungs, brain, skin and genitalia. The visceral lesions are abscesses while the superficial lesions manifest as ulcers. • Severe morbidity in serious infections can lead to death. • Flask shaped ulcer. Histologically when one examines the intestine in a case of E. histolytic, the lesion of the intestine is characteristically described as a flask

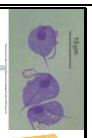
		shaped ulcer. The parasite buries initially perpendicular to the surface of the mucosa and then buries laterally in the submucosa causing overhanging mucosal edges and a flask shape.
Slide 16	**Diagnosis** • Microscopic identification of cysts and trophozoites in the stool – Characteristics: bulls-eye nucleus, ingested red blood cells, chromatin bar • Differentiation from other amoebae based on morphologic characteristics of the cysts and trophozoites. – *Entamoeba dispar* (non-pathogenic) vs. *E. histolytica*, based on isoenzymatic, immunologic, or molecular analysis. 	The diagnosis of E. histolytica is by microscopic examination of the stool. The top figure on the right shows the cyst with the characteristic bulls-eye nuclei. The central bulls eye is the karyosome. Sometimes a rod-like structure is seen which is a chromatin rod (red arrow). It may also show ingested red blood cells. The lower figures show the trophozoites with irregular shape. The trophozoites can look similar to another species of Entamoeba called E.dispar which is not pathogenic and the differentiation is based on isoenzymatic, immunologic or molecular analysis. CDC pics and www.ksu.edu/.../546tutorials/PROTFIG13.JPG
Slide 17	**Treatment** • Asymptomatic- only carry cysts – Metronidazole (Flagyl) – Iodoquinol – Diloxanide furoate (Furamide) if passing cysts (not in U.S.) – Paromomycin • Symptomatic – Acute amebic dysentery ▪ Emetine HCL – Liver abscess ▪ Flagyl ▪ Tinidazole or Ornidazole (not in US) – Followed by treatment with iodoquinol, paromomycin, or diloxanide furoate 17	The most important drug to remember in the treatment of asymptomatic and symptomatic amoebiasis is metronidazole. Metronidazole does not kill cysts. Outside of the US the drug Diloxanide furoate is used as cysticidal agent for treatment of amebiasis so that both cysts and trophozoites are killed.

Slide 18	**Prevention** • Safety of drinking water supplies – Cysts are fairly resistant to chlorination of drinking water • Environmentally stable cysts – Heat water above 50°F – Freezing kills cysts • Traveler precautions	The prevention of amoebiasis is centered on the consumption of safe water. The cysts can be resistant to chlorination so the water should be filtered even if chlorinated. The best means is probably boiling of the water prior to consumption as the cysts are destroyed at temperatures above 50^0 C. Freezing can also kill the cysts. Travelers should bear in mind that infected individuals and carriers can come in contact with the drinking water and contaminate it.
Slide 19	**Outbreaks** • World's Fair, Chicago (1933) – >1400 cases, 100 deaths • Los Angeles, CA. (1983) – 38 patients over the course of 3 months were diagnosed, in comparison to a previous frequency of about 1 case per month. • Tbilisi, republic of Georgia (1998) – A case-control study indentified 177 cases, but outbreak was widespread may have affected 84,000–225,000 people. • Emerging in Japan – Male homosexuals and mentally handicapped persons in institutions. ▪ 500–600 cases of amebiasis reported annually, with 3-4 deaths	This slides lists some of the outbreaks of amoebiasis that have been previously reported. US has had outbreaks as late as the eighties. Homosexual transmission has now emerged as another mode of transmission and also outbreaks in prisons and institutions for the mentally disabled.
Slide 20	***Giardia lamblia (Giardia intestinalis)*** • Obligate parasite that infects numerous mammalian hosts • Fecal-oral transmission through food and water • Distributed worldwide, more prevalent in warm climates, and in children. • Prevalence 3-7% in developed countries • Average prevalence of 20% in undeveloped countries • **The most frequent cause of non-bacterial diarrhea in U.S.** • During 2009–2010, the total number of reported cases of giardiasis increased slightly from 19,403 for 2009 to 19,888 for 2010. A larger number of case reports were received for children aged 1–9 years than with other age groups. The number of cases peaked annually during early summer through early fall. (CDC) • Risk groups in the US: travelers, children in day care, homosexual men Flagellated with ventral sucker disk (for attachment)  	Giardia lamblia also called Giardia intestinalis is an obligate parasite that infects numerous mammalian species. The transmission is feco-orally through contaminated water. It has a worldwide distribution with higher prevalence in developing countries. In the US the latest CDC surveillance report indicates an increase of 1.9 % in 2010 over the previous year. The cases tend to peak in early summer through early fall. Children aged 1-9 were the ones with highest incidence followed by those in the 35-45 years age group. In both age-groups males had the highest incidence. The top figure shows a trophozoite of G.lamblia with a large anterior ventral suction cup with which it attaches to the intestinal mucosa. The lower figure shows a carpet of G. lamblia attached to the intestinal mucosal surface.

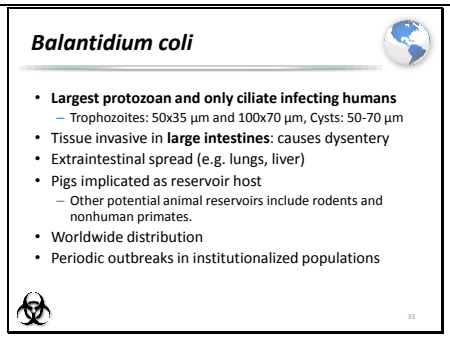
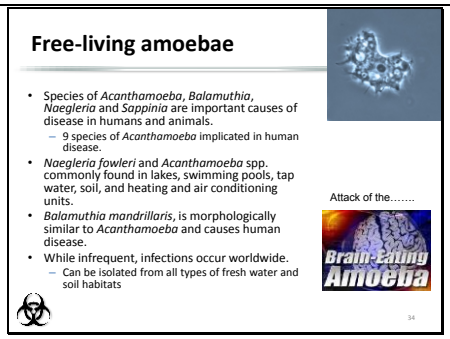
Slide 21		The graphic shows the lifecycle of Giardia lamblia. Humans get infected by consuming the cysts. The trophozoites excyst in the small intestine and get attached to the mucosal surface. They multiply by binary fission as seen in the lower box. The diagnosis is by detection of the characteristic cysts and occasionally trophozoites.
Slide 22		The infection causes an acute disease that can occur upto 2 weeks after infection but on average manifest within 1 week. It can last upto 1-3 weeks It is characterised by abdominal cramps, diarrhoea, bloating, nausea and steatorrhoea which is fatty deposits in the stools. The attachment of the parasites by their suction plates damages the microvilli of the small intestine. The large number of parasites cause malabsorption of food. Symptoms are due small intestinal mucosal inflammation. Some individuals have asymptomatic carriage and they could be responsible for outbreaks. In Giardia, an immune evasion strategy occurs when the organism undergoes encystation. As a result of this an individual who may have partial protection due to prior exposure is unable to prevent infection due to different antigens the second time around.
Slide 23		The diagnosis of the infection is usually done by the identification of the characteristics cysts and trophs in feces. Examination of the duodenal aspirate can reveal trophozoites. Antigen detection tests by enzyme immunoassays and also detection of parasites by immunofluorescence assays. Treatment is by metronidazole, Tinidazole or nitazoxanide in children.

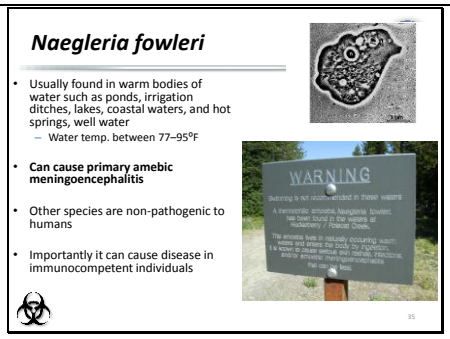
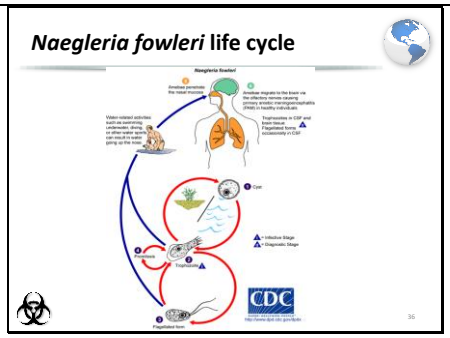
Slide 24	**Prevention**  • Proper hygiene and disposal of waste • Avoid drinking water from sources associated with outbreaks • Avoid swallowing water while swimming in public pools, water parks • Avoid livestock and exotic pets, zoonotic transmission • In day care centers: wash hands and proper disposing of diapers • Hikers and backpackers: boil, filter, chemically treat water • Water filtration (moderate Chlorine resistance)  http://www.cdc.gov/healthywater/pdf/swimming/resources/giardia-factsheet.pdf  	Prevention of Giardia is focused on hygiene and sage disposal of waste. Avoid drinking water from sources associated with outbreaks and from congregations of humans especially in swimming pools and water parks. Livestock and exotic pets could act as reservoirs for Giardia resulting in zoonotic transmission. In day care centers it is essential that the soiled diapers are properly disposed and that caretakers and children wash their hands. Hikers and backpackers should boil, filter, chemically treat water prior to consumption. Water filtration is essential as the organism has moderate chlorine resistance.
Slide 25	**Major Outbreaks**  • Europe – Sweden 1986- >1400 cases – Norway 1994- >1300 cases • U.S. – Colorado (Vail-1978): 5,000 cases – Rome, N.Y. (1974-1975): 4800-5300 cases – Bradford, PA. (1979): 3500 cases – Berlin, N.H. (1977): 7000 cases – Florida (1996): 77 cases – The most recent is in Niagara reported in February 2013. with about 18 cases.  25	This slide show some of the outbreaks of Giardia. The most recent was in Niagara in February 2013 with about 18 cases.
Slide 26	***Trichomonas vaginalis***  • **Causes the most prevalent non-viral sexually transmitted disease worldwide** • Only known host is humans • Worldwide prevalence, but higher prevalence among persons with multiple sexual partners or other venereal diseases • **No cyst stage; sexual transmission of trophozoites during intercourse** • Found in vagina and urethra of women and prostate, seminal vesicles, urethra of men • Other non-pathogenic species: *T. hominis, T. tenax* – ***Trichomonas* is the most common parasitic infection in the U.S., accounting for an estimated 7.4 million cases per year. (CDC)** Characteristic multiple flagella at top and axostyle on side   26	The next parasite is Trichomonas vaginalis. It is the cause of the most common non viral sexually transmitted disease. Its only known host is humans. It occurs worldwide but is most commonly seen in promiscuous individuals or individuals with other STDs. This parasite has no cyst stage and the transmission from one individual to another is during sexual intercourse. It is found in the vagina and urethra of women and the seminal vesicles and urethra of men. T. hominis and T.tenax are non-pathogenic species. It is the most common parasitic infection in the United States.

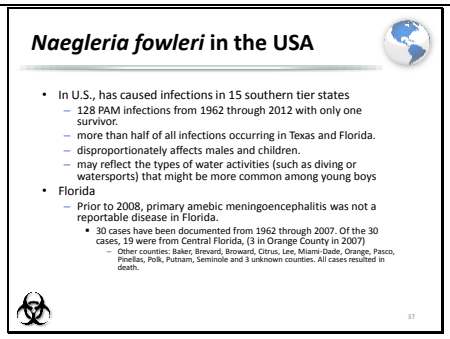
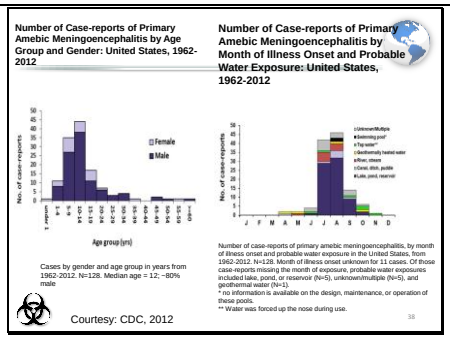
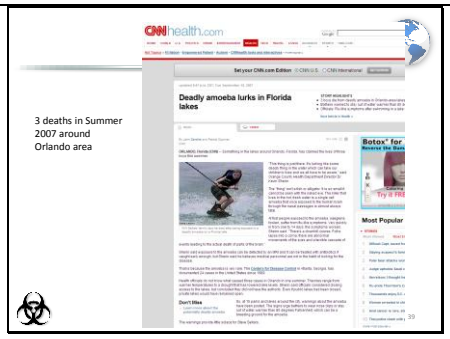
Slide 27		The table from **Baseline report on global sexually transmitted infection surveillance 2012**, of WHO published in 2013 shows an increase in Trichomonas vaginalis infections by 11.3 percent in the three years from 2005 to 2008 worldwide. In the US, the latest 2013 report by CDC shows that for 2008, the incidence rate for T. vaginalis was 1.09 millions with a prevalence of 3.7 millions. (**Incidence, Prevalence, and Cost of Sexually Transmitted Infections in the United States, February 2013)**
Slide 28		The graphic shows the lifecycle of T. vaginalis. It is the simplest lifecycle in my opinion. There is no cyst stage, no intermediate host or intermediate stages. The multiplication is by binary fission. The parasite does not survive well in external environment. Transmission between humans is by sexual intercourse.
Slide 29		Now lets look at the clinical features. In women, the infection is persistent and causes a white vaginal discharge which sometimes has a fishy odor. It may be accompanied with some vulvar or cervical ulcerative lesions, abdominal pain, pain on urination (dysuria) and during sexual intercourse (dyspareunia). The erosions on the genito-urinary mucosal surface increase the risk of HIV infection. Pelvic Inflammatory disease can damage in the ovarian (fallopian) tubes and tissues around the uterus and ovaries. Also, there is an increased risk of cervical cancer and preterm births due to premature rupture of amniotic membranes and low birth weight infants. In men, the infection is generally asymptomatic. Sometimes it infects the prostate gland and seminal vesicles. As in women, it increases the risk for HIV infection and is a common cause of non-gonococcal urethritis. It may cause male infertility.

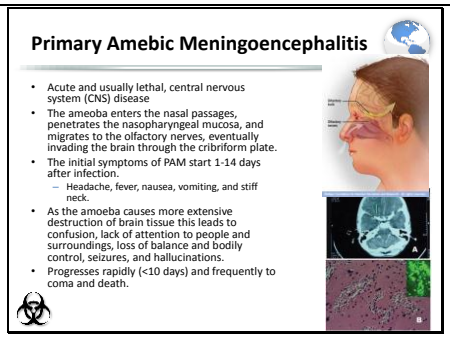
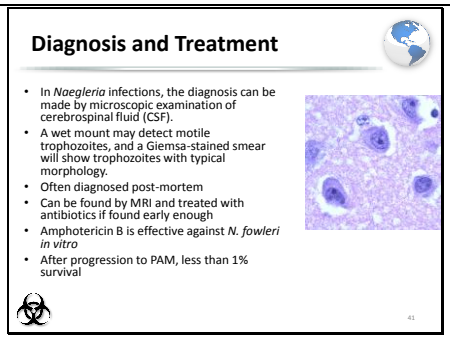
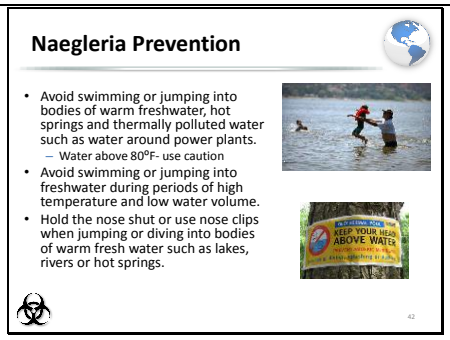
Slide 30	**Trichomoniasis: Diagnosis/Rx** • Clinical presentation: discharge, strawberry cervix • In men, anterior urethral or prostatic secretions should be examined. • Microscopic examination of vaginal and urethral secretions can establish the diagnosis by detecting actively motile organisms. • Direct immunofluorescent antibody staining is more sensitive than wet mounts, but technically more complex. • PCR assays **Treatment** • Metronidazole and tinidazole; therapy is usually highly successful. • Strains of *Trichomonas vaginalis* resistant to both drugs have been reported.   	Clinically, infection in women presents as vaginal discharge with strawberry cervix which is punctate red lesion seen on cervical mucosal surface in women. Men may have urethral discharge and sometime redness on the urethral meatus or glans penis. The lab diagnosis of trichomoniasis is done by the wet mount preparation of vaginal, urethral or prostatic discharge/fluid. The trichomonas are motile organisms and easily identified. They can also be detected using immunofluorescent stains. Treatment should be implemented under medical supervision, and should include all sexual partners of the infected persons. It is easily done by metronidazole. Some resistance to metronidazole has been reported.
Slide 31	**Prevention** • Abstain from sexual contact, or to be in a long-term mutually monogamous relationship with a partner who has been tested and is known to be uninfected. • Condom use for as long as both partners are infected • Any genital symptom such as discharge or burning during urination or an unusual sore or rash=consult a health care provider immediately. • Possible transmission via contaminated towels, washcloths, clothing	Prevention- This is best by abstinence. Monogamous relationships with a partner who has been tested and is known to be uninfected. Condoms should be used for as long as both partners are infected. Any genitourinary symptoms such are burning, pain or redness should be reported to a health care provider and consultation sought immediately. The infection could potentially be transmitted via contaminated towels, washcloths or clothing.
Slide 32	Comparing, *Giardia*, *Trichomonas* and *E. Histolytica* Review, anyone?   	This is a slide that reviews in a glance the protozoal parasites that we have covered so far. E.histolytica is the only amoeba in the group, T.vaginalis has no cyst stage, Giardia affects animals as well as humans, T.vaginalis is sexually transmitted and results in vaginitis.

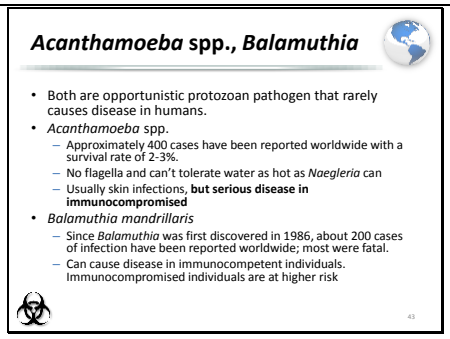
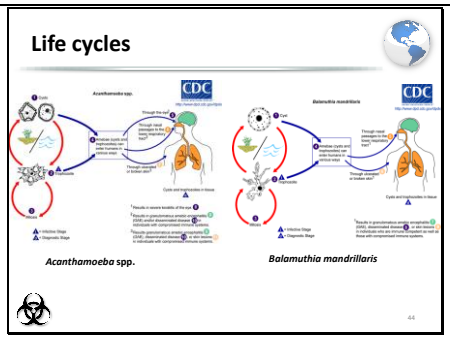
	G. lamblia	*E. histolytica*	*T. vaginalis*
Morphology	Flagellate	Ameba	Flagellate/Ameba
Life forms	Trophozoite Resistant Cyst	Trophozoite Resistant Cyst	Trophozoite only No Cyst form
Host	Many mammals including humans	Humans Only	Humans Only
Transmission	Ingestion of Cyst	Ingestion of Cyst	Sexual Intercourse
Disease Manifestation s	Diarrhea/ Dysentery	Diarrhea/ Dysentery	Vaginitis

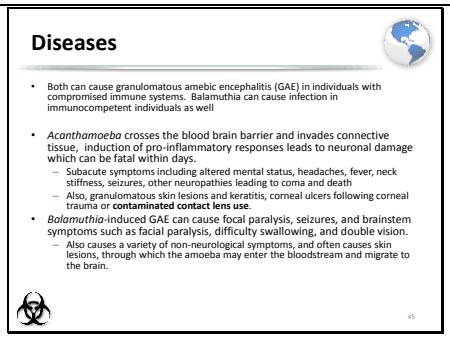
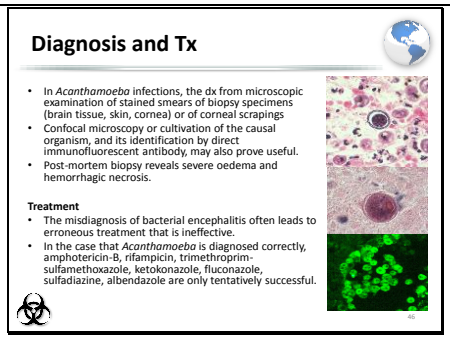
Slide 33		Balantidium coli is the only ciliated organism that infects humans. Cilia are the hairlike structure on the outside of its coat. It is a large micro-organism. It is worldwide in distribution and affects human mainly causing colitis and dysentery. It can spread to lungs and liver like E. histolytica. Pigs and some other animals like rodents are its other reservoirs. Periodic outbreaks are reported in institutionalised individuals.
Slide 34		The free living amoebas are organisms that are free living in the environment and cause disease in humans and animals upon entering the body. Of interest are the organisms belonging to four genera- Acanthamoeba, Naegleria, Balamuthia and Sappinia. Acanthameba has nine species that have been implicated in human disease. Both Naegleria and Acanthameba are found in lake water, swimming pools, tap water and heating and air conditioning units. It is for this reasons one should not wash contact lenses with tap water. Balamuthia mandrillaris is similar in morphology to Acanthameba and cause human disease. It is commonly found in the soil. Although infections with these organisms are rare, they can be fatal and the organism can be isolated from soil and surface fresh water sources. Ubiquitous and world wide

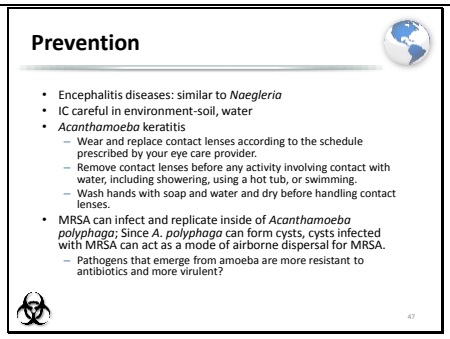
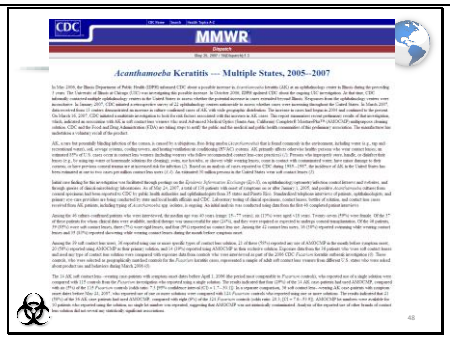
Slide 35		Naegleria fowleri is usually in warm surface water sources such as ponds, irrigation ditches, hot springs and wells. It prefers a water temperature between 77-95^0 C. It is therefore common to have reports of this infection in the summer months when people tend to spend more time outdoors on lakes and in ponds. Florida has had few case reports that have been fatal. The other species of Naegleria are not pathogenic to humans. Its only the fowleri species that is pathogenic. The amoeba enters the body through the nose while swimming, diving and it attaches to the olfactory nerves and travel upwards to enter the brain through the cribriform plate to cause infection and inflammation of the meninges and the brain – meningoencephalitis. Infection with *Naegleria is most common during the summer months of July, August, and* September. Importantly Naegleria can cause infections in immunocompetent individuals.
Slide 36		The graphic depicts the life cycle of N.fowleri. The ameba can exist in trophozoite form or cyst form in nature and does not need a host to do so. It can attain a flagellated form and this is freely motile. The trophozoite form divides by a process called promitosis to multiply. The flagellated form or trophozoite upon entry into humans can actively penetrate the nasal mucosa and then ascend along the olfactory nerves to cross the cribriform plate and cause meningoencephalitis. The diagnosis is usually done at autopsy by detection of the trophozoites under the microscope. Alternates between flagellated and amoeboid form

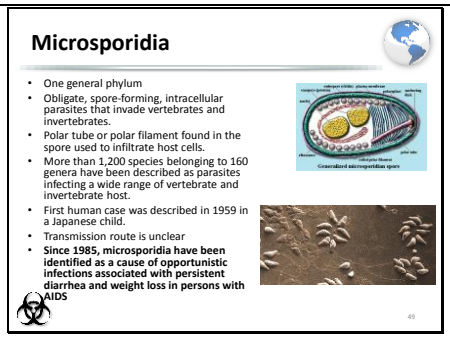
Slide 37		Let's now look at the cases of Primary Amoebic Meningoencephalitis (PAM) in the US. Since 1962 there have 128 cases with only one survivor mainly in the southern tier states. More than half of all infections occur in Texas and Florida. Males and children are affected more and probably as a result of increased water activities in the summer such as diving. In Florida, prior to 2008, PAM was not a reportable disease. 30 cases have been documented from 1962 through 2007. Of these 30 cases 19 were from C.Florida. All resulted in deaths.
Slide 38		The left graphs show the number of cases of PAM age-wise and gender-wise. For the years 1962-2012. The right graph shows most of the cases occur in summer and the exposure is commonly lakes and ponds.
Slide 39		This is an example of the media coverage of an outbreak that occurred in 2007 with three cases around Orlando. So if any of you are into recreational water activities like wakeboarding, jet skiing etc., be aware and watch out for signs that indicate the presence of N. fowleri and the risk of PAM. Be very careful.

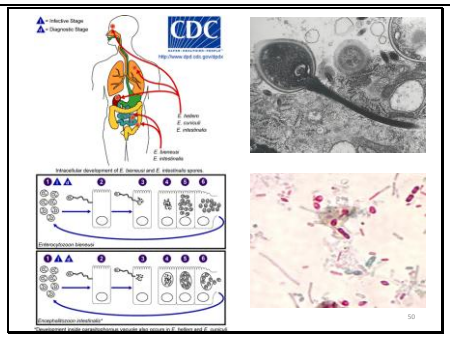
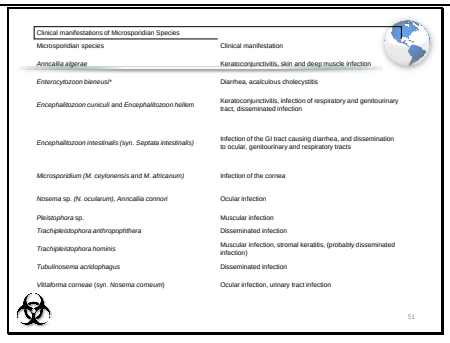
Slide 40		Primary Amebic Meningoencephalitis (PAM) is acute and is usually fatal Central Nervous system disease. The ameba enters the nasal passages, penetrates the nasopharyngeal mucosa, and invades the olfactory nerves to travel up through the cribriform pates of skull and infect the meninges and brain. The initial symptoms of PAM begin usually 1-14 days after infection. Headache, fever, nausea, vomiting and stiff neck due to inflamed meninges. With progressive brain tissue damage, there is confusion, inattention to people and surroundings, loss of balance and bodily control, seizures and hallucinations. It is rapidly progressive and frequently leads to coma and death.
Slide 41		The diagnosis of Naegleria infections can be done by detection of the motile trophozoites in wet mounts of cerebrospinal fluid. The CSF can be stained with Giemsa to detect the amoebae. However, usually the diagnosis is done post mortem at autopsy. In vivo MRI can detect PAM and treatment with antibiotics can be initiated in very early diagnosis. N.fowleri is sensitive to Amphotericin B in vitro. Once PAM sets in prognosis is grim.
Slide 42		Prevention of Naegleria is focused on preventing the entry of water into the nasal tract especially when engaged in recreational water activities. Avoid swimming or jumping into bodies of warm freshwater, hot springs, and thermally polluted water as seen around power plants. Hold the nose shut or use nose clips when jumping or diving into bodies of warm fresh water. Always look out for signs such as one shown in the lower figure prior to engaging in water recreation activities.

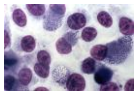
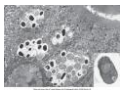
Slide 43		Both are opportunistic organisms and infection usually occurs in patients with an immunodeficiency, diabetes, malignancies, malnutrition, systemic lupus erythematosus, or alcoholism. These infections are otherwise rare in humans. Acanthameba: About 400 cases of amebic encephalitis due to Acanthameba have been reported worldwide with a survival rate of 2-3 %. The Acanthameba have no flagella like Naegleria and also do not tolerate warm water temperatures. They usually cause skin infections which can be serious in immunocompromised individuals. Since *Balamuthia* was first discovered in 1986, about 200 cases of infection have been reported worldwide. Most of them have been fatal. It is extremely rare and is seen mostly in immunocompromised individuals. It can affect immunocompetent individuals as well.
Slide 44		This slide shows the life cycles of Acanthameba and Balamuthia. In both they have cyst and trophozoite forms and humans get infected upon exposure in surface fresh water activities. In Acanthameba the infection results in granulomatous inflammation called granulomatous amebic encephalitis especially in immunocompromised individuals. It can cause infection of the cornea in contact lens wearers or upon ocular contact with contaminated water in immunocompetent individuals. It is an intractable infection and difficult to treat. It can lead to corneal opacification necessitating corneal transplant. It can even lead to blindness from loss of the eye. Balamuthia is mainly a soil ameba. It can cause disease in both immunocompetent as well as immunocompromised individuals. The exposure routes are usually through cuts and wound of skin or by breathing in soil during garden work. Swimming has yet to be reported to be associated with Balamuthia granulomatous encephalitis. The time from infection to diseases can be many weeks to sometimes months.

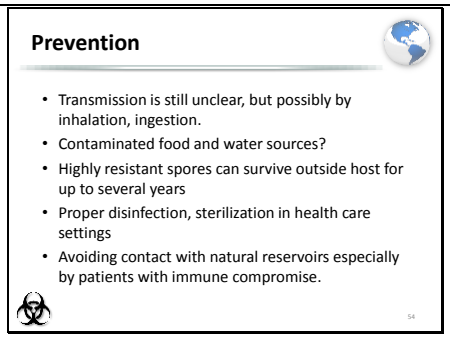
Slide 45		Both Acanthameba and Balamuthia cause granulomatous amebic encephalitis in immunocompromised individuals. Acanthameba infection is very rapid and sets up inflammation and tissue destruction rapidly causing fatality. Sub-acute symptoms can range from headache, fever, neck stiffness, seizures, other neuropathies leading to coma and death. It the eye, it causes keratitis following trauma or contaminated contact lens use. In the skin it cause granulomatous ulcers. *Balamuthia mandrillaris* may enter the body through the lower respiratory tract or through open wounds. Upon introduction, the amoebas may form a skin lesion, or migrate to the brain. In HIV/AIDS there may be disseminated infection. The Balamuthia induced granulomatous encephalitis can cause focal paralysis, seizures and brainstem features such as facial paralysis, dysphagia and diplopia. It also causes various non-neurological symptoms, often- skin lesions through which the amebae enter the bloodstream and migrate to the brain.
Slide 46		Microscopic examination of samples from regions of the body infected by the parasite usually leads to the diagnosis. For Acanthameba, in skin and corneal infections, skin biopsy or corneal scrapings can help in diagnosis after staining. IN the patient, confocal microscopy can reveal the present of trophozoites and cysts in the cornea. Post mortem exam reveals severe swelling and hemorrhagic brain necrosis. Treatment : Acanthameba infections are difficult to treat. The keratitis is very persistent and the currently available drugs like brolene sometimes take weeks to effect a cure. Many times the patients ends up getting a corneal transplant for persistent infection and corneal opacification. There have been cases, where the corneal graft has ended getting infected from the host tissue. Like wise brain infections are also very difficult to treat. If diagnosed early treatment can be initiated with Amphotericin B, rifampicin, trimethoprim-sulphamethoxazole, ketoconazole, fluconazole, sulphadiazine. Some patients have recovered following CNS infection with proper treatment.

Slide 47		The prevention of Acanthameba and Balamuthia infections is similar to the Naegleria especially the immunocompromised individuals that are at increased risk. With Acanthameba, there is risk of keratitis in persons using contact lenses. So it is essential to practice safe contact lens handling practices. To wash hands with soap and water prior to inserting and removing contact lenses and not to contaminate them by using tap water or any other liquid except the contact lens cleaning solution. Interestingly A. polyphaga can harbor MRSA. Also ,it has been theorised that MRSA from such amebae are more antibiotic resistant. So with the ubiquitous presence of the amebae in the environment, the risk of highly resistant MRSA emerging has been predicted.
Slide 48		This is an actual report of an outbreak of Acanthameba keratitis that occurred from 2005 to 2007 leading to a voluntary recall of Complete MoisturePlus Multi Purpose Solution manufactured by Advanced Medical Optics of Santa Ana, CA due to contamination with Acanthameba. Usually, *Acanthamoeba* keratitis infections occur in approximately 2 out of every 1 million contact lens users in the United States each year. However, in a multi-state investigation to evaluate a recent increase in *Acanthamoeba* keratitis cases, CDC determined that the risk of developing AK was at least seven times greater for those consumers who used Complete MoisturePlus solution versus those who did not.

Slide 49	 	The final parasite for our consideration in this lecture is Microsporidia. Microsporidia is a phylum of eukaryotes that contains almost 160 genera. Related to fungi, they are obligate intracellular organisms that spread among hosts through a spore stage. The figure on the right at the tops shows a schematic microsporidian spore. There is a polar tube at one end that is used to invade the host cells. More than 1200 species the different genera have been described in a very wide variety of vertebrate and invertebrate hosts. The first human case was described in a Japan in a child in 1959. The mode of infection is unclear. Since 1985, microsporidia have been identified as a cause of opportunistic infections associated with persistent diarrhoea and weight loss in persons with AIDS. Image Credits: http://images.google.com/imgres?imgurl=http://www.stanford.edu/class/humbio103/ParaSites2006/Microsporidiosis/microsporidia1_files/image004.jpg&imgrefurl=http://www.stanford.edu/class/humbio103/ParaSites2006/Microsporidiosis/microsporidia1.html&usg=__z6cd5f-Ft81vlGP_X3DJeko4sWU=&h=296&w=440&sz=39&hl=en&start=1&um=1&tbnid=sB0K8cKUUa8o1M:&tbnh=85&tbnw=127&prev=/images%3Fq%3Dmicrosporidia%26hl%3Den%26client%3Dfirefox-a%26rls%3Dorg.mozilla:en-US:official%26sa%3DN%26um%3D1 http://www.cdc.gov/eid/content/16/2/335.htm

Slide 50		The infective form of microsporidia is the resistant spore and it can survive for a long time in the environment . The spore extrudes its polar tubule and infects the host cell . The spore injects the infective sporoplasm into the eukaryotic host cell through the polar tubule . Inside the cell, the sporoplasm undergoes extensive multiplication either by merogony (binary fission) or schizogony (multiple fission) . This development can occur either in direct contact with the host cell cytoplasm (e.g., *E. bieneusi*) or inside a vacuole termed parasitophorous vacuole (e.g., *E. intestinalis*). Either free in the cytoplasm or inside a parasitophorous vacuole, microsporidia develop by sporogony to mature spores . During sporogony, a thick wall is formed around the spore, which provides resistance to adverse environmental conditions. When the spores increase in number and completely fill the host cell cytoplasm, the cell membrane is disrupted and releases the spores to the surroundings . These free mature spores can infect new cells thus continuing the cycle. The figure on the top at right shows transmission electron micrograph of a microsporidian spore with an extruded polar tubule inserted into a eukaryotic cell. The spore injects the infective sporoplasms through its polar tubule. The lower right figure shows Spores of *T. acridophagus* in BAL specimens, stained with Chromotrope 2R stain. (CDC, Nov 2013)
Slide 51		This table gives a list of human pathogenic microsporidia. This table is not for memorisation.

Slide 52	**Wide range of disease** • Occurs mainly, but not exclusively, in severely immunocompromised patients with AIDS. • **Chronic diarrhea and wasting are the most common symptoms of microsporidiosis** • Disseminated infection is characterized by symptoms of cholecystitis (inflammation of the gallbladder), renal failure, respiratory infection, headache, nasal congestion, ocular pain and sinus involvement. • Respiratory infection may cause cough, dyspnea (labored breathing) and wheezing. • With ocular infection, symptoms range from foreign body sensations, eye pain, light sensitivity, redness, excessive tearing or blurred vision. • Finally, infections of the brain or other nervous tissue cause seizures, headache and other symptoms depending the precise area of infection. • Diffuse myositis and muscle necrosis. 52	The microsporidia cause a wide range of clinical disease symptoms. They are mainly significant as opportunistic agents in patients with decreased immunity such as those with AIDS. Chronic diarrhoea and wasting are the most common symptoms. Dissemination leads to inflammation of the gall bladder, headache, nasal congestion, ocular pain and sinus involvement. Infection of the respiratory tract leads to cough, dyspnoea and wheezing while infection of the eye leads to pain, decreased vision, foreign body sensation, redness and watering. Infections of the brain and nervous tissue can cause seizures, headache and other symptoms depending on the locus of infection. Myositis and muscle necrosis.
Slide 53	**Diagnosis and Treatment** • DX: Microscopic ID-light and TEM, IFA, PCR • Ocular microsporidiosis-oral albendazole plus topical fumagillin. – Corneal infections with *V. corneae* often do not respond to chemotherapy and may require keratoplasty. • Oral fumagillin has been effective to treat *Enterocytozoon bieneusi* infections, but it has been associated with thrombocytopenia. • Albendazole for gastroenteritis caused by *Encephalitozoon intestinalis* and to treat disseminated microsporidiosis (various species) and skin and deep muscle infection (*Brachiola algerae*). • Immune restoration to CD4 cell count >100 cells/mm3 is associated with resolution of symptoms of enteric microsporidiosis in patients with HIV.   53	Most reports addressing prevalence of microporidiosis are based on coprologic or PCR diagnostics, and the serologic screening of humans for microsporidia infection has mostly been limited to species that can be cultured in vitro. The diagnosis of microsporidiosis is mainly based on microscopic examination of tissues using light microscopy and stains such as Hand E and some special stains such as Warthin-Starry stain, Calcofluor white etc. They can also be diagnosed using transmission Electron Microscopy or fluorescent antibodies. Polymerase chain reaction has also been used for diagnosis. Systemic infection has been treated with oral albendazole or fumagillin. Depending on site of infection. Ocular infection treated with topical fumagillin and sometimes with oral fumagillin along with albendazole. Disseminated, GI and skin and muscle microsporidiosis treated with oral albendazole. Finally, Immune restoration to CD4 cell count >100 cells/mm3 is associated with resolution of symptoms of enteric microsporidiosis.

Slide 54	 	Prevention of infection by microsporidia is still unknown given we still do not have a complete understanding of their transmission to humans. Possible sources are contaminated food and water. The spores are highly resistant and survive outside the human body in the environment. Proper disinfection and sterilisation in healthcare settings is important. The identification of microsporidia in human body fluids necessitates the following proper hand washing protocol to prevent transmission. Also, certain species of domestic and wild animals and birds have been identified as naturally infected with microsporidia. For example, it is now known that some domestic and wild animals may be naturally infected with the following microsporidian species: *E. cuniculi*, *E. intestinalis*, *E. bieneusi*. Birds, especially parrots (parakeets, love birds, budgies) are naturally infected with *E. hellem*. *E. bieneusi* and *V. corneae* have been identified in surface waters, and spores of *Nosema* sp. (likely *A. algerae*) have been identified in ditch water. *Tubulinosema acridophagus*, an insect parasite, has recently (2012) been implicated in two cases of disseminated microsporidiosis. The preventive methods sections will likely get updated as more knowledge is gained over time about these opportunistic infections.