Chapter 1

Introduction

Food Microbiology is the area of Microbiology that studies microorganisms that inhabit, create, or contaminate food as well as causes food spoilage and food borne diseases. Food borne diseases are a widespread and increasing Public Health problem in developed and developing countries. (1). Approximately 76 million cases of foodborne diseases occur each year in the United States of America. (2). Food borne diseases are caused by eating food or drinking beverages contaminated by bacteria, viruses, parasites, and fungi. Harmful chemicals also cause foodborne diseases if they contaminate food while they are being harvested or during processing.

The toxins produced by microorganisms can cause food borne illnesses if they are not destroyed in the cooking process, that is, if they are not heat labile. Raw foods are a popular source of foodborne diseases because they may not be sterile, for example meat and poultry. Sea food can become contaminated through harvesting and processing. Raw vegetables irrigated with ground water that may be contaminated with enteric viruses can be associated with foodborne viral disease outbreaks. (3). Most foodborne diseases are rarely diagnosed because they have mild effects. Microorganisms and their toxins play the prominent role in foodborne diseases. Mushroom poisoning is not considered microbial because of its size, but the situation is unique because mushrooms are classified as fungi.

Chapter 2

Bacteria in Foodborne Diseases

The most commonly found bacteria that cause food borne diseases are Yersinia entercolitica, Vibrio vulnificus, Staphylococcus aureus, Campylobacter, Listeria monocytogenes, Salmonella, Shigella, Escherichia coli 0157.H17, Clostridium botulinum, Clostridium perfringens, Bacillus cereus, and Brucella spp.

Yersinia entercolitica

Yersinosis is an infectious disease caused by a bacterium of the genus Yersinia. (4). In the United States, most human illnesses are caused by one species, Yersinia entercolitica. (4). Yersinia entercolitica is a member of a family of rod shaped bacteria. Other species of bacteria in this family include Yersinia pseudotuberculosis, which causes illnesses similar to Yersinia entercolitica, and Yersinia pestis, which causes plague. (4). Only some strains of Yersinia entercolitica cause illnesses in human. The most pathogenic strains of Yersinia entercolitica associated with human yersinosis belong to the bioserotypes 1B/0:8, 2/0:5,27, 2/0:9, 3/0:3 and 4/0:3. (5). The major animal reservoir for Yersinia entercolitica are domestic animals such as pigs, dogs, cows, sheep and cats; wild animals such as mice, monkeys, deer, boar, snail and foxes. (5). Food samples such as milk and milk products, meat and poultry products, oysters, mussels, shrimp, blue crab, fish, stew mushrooms, and vegetables-cabbage, celery and carrots are known to contain Yersinia entercolitica. (5). Yersinia entercolitica was isolated from environmental samples such as sewage water, soil, fodder and slaughter houses. (5).

Infection with Yersinia entercolitica is associated with eating contaminated food, food products, some sea food, or contact with environmental samples such as sewage, soil, fodder, or slaughter houses. The preparation of raw pork intestines may be particularly risky. (3:6). Yersinia entercolitica is frequently reported as a zoonotic gastrointestinal disease after Campylobacteriosis and Salmonellosis in many developed countries, especially in temperate zones. (7). Within developed countries, incidences of Yersinosis food borne disease appeared to be lower in the United States than many European countries. (8:9:10). On the other hand, high prevalence of gastrointestinal illnesses including fatal cases due to

yersinosis is also observed in many developing countries like Bangladesh, Iran, Iraq, and Nigeria, which indicates major underlying food safety problems in low and middle income countries. (11: 12: 13: 14: 5).

Worldwide infection with Yersinia entercolitica occurs most often in infants and young children with common symptoms like fever, abdominal pain, and diarrhea, which is often bloody. (6:5). The incubation period for Yersinia entercolitica is 4-7 days and the chronic effects are reactive arthritis and Graves-Basedow thyroiditis. (6). Uncomplicated cases of diarrhea due to Yersinia entercolitica are usually resolved on their own without antibiotic treatment. However, antibiotics such as aminoglycosides, doxycycline, trimethoprim-sulfamethoxazole, or fluoroquinolones may be useful in treating severe and complicated infections.

Vibrio vulnificus

Vibrio vulnificus is a species of gram-negative, motile, curved bacterium that is part of Vibrio genus and the Vibrionaceae family.(15). The vibrionaceae family also include Vibrio cholera (which is rare in the United States), and Vibrio parahaemolyticus, both of which cause acute gastrointestinal illness characterized by severe diarrhea. (15). Four other species (Vibrio mimicus, Vibrio hollisae, Vibrio fluvialis, and Vibrio furnissii can cause gastroenteritis. Vibrio vulnificus is common in warm water and thrives in water temperatures greater than 68F (20 degrees Centigrade). (16: 15). The organism is not associated with pollution or fecal waste. (15). Some strains of these species produce known toxins, but the pathogenic mechanisms is not readily understood. (17). The ecology of detection and control methods for all sea food borne Vibrio pathogens are essentially similar. (17).

Vibrio vulnificus is found in all coastal waters of the United States. (15). Most Vibrio vulnificus infections are attributed to consumption of raw or undercooked seafood, particularly oysters harvested in the Gulf of Mexico during the summer. (18: 16). Because these oysters are shipped throughout the United States, infections are not limited to endemic areas. (19). Proper cooking methods readily kill Vibrio vulnificus. (15).

Approximately 25 percent of Vibrio vulnificus infections are caused by a direct exposure of an open wound to warm sea water containing the organism. (15). Exposure typically occurs when the patient is participating in water activities such as boating, fishing or

swimming. (15). Infections are occasionally attributed to contact with raw seafood or marine wildlife. (18). Vibrio vulnificus is one of the few foodborne illnesses with an increasing incidence. (15). The Center for Disease Control and Prevention estimates that the average annual incidence of all Vibrio infections increased by 41 % between 1995 and 2005. (20).

The symptoms of Vibrio vulnificus infection by ingestion are vomiting, diarrhea, abdominal pain, and hemorrhagic and necrotic bullae ecchymoses. (16: 15). In immunocompromised persons particularly those with chronic liver disease, Vibrio vulnificus can infect the blood stream causing severe and life threatening illness which is characterized by fever and chills, and decreased blood pressure (septic shock), and blistering skin lesions . (16: 15). Vibrio vulnificus infections are fatal about 50% of the time. (16). Vibrio vulnificus infections of the skin can also occur when open wounds are exposed to warm seawater. (16: 15). These infections may lead to skin breakdown and Ulceration. (16). Individuals who are immunocompromised are at higher risk for the invasion of the microorganism into the blood stream and potentially fatal complications. (16). Vibrio vulnificus infection is treated with antibiotics such as Doxycycline or Ceftazidime. (16: 15).

Staphylococcus aureus

Staphylococcus aureus is a common cause of bacterial foodborne disease throughout the world. Staphylococcus aureus food poisoning is often caused when the food handler contaminates food products that are served or stored at room or refrigerated temperature. (21). Examples of foods normally contaminated are desserts (especially custards and cream-filled or topped desserts), salads- particularly those containing mayonnaise such as tuna salad, potato salad, and macaroni salad, poultry and egg products, and casseroles. (21). Staphylococcus aureus may occur as a commensal or human skin, nose and throat, and occurs in about a third of the population. (22). It is from these locations that food handlers can cause contamination. Direct skin contact with food and sneezing may be common methods. The bacteria produce a toxin in the food and the toxin causes most of the symptoms. Depending on the strain, Staphylococcus aureus, is capable of secreting several toxins, which are categorized into three groups-pyrogenic toxins super antigens which include the staphylococcal enterotoxins that cause food poisoning, exfoliated

toxins and other toxins that act on cell membranes such as alpha toxins, beta-toxins, delta-toxins and several bicomponent toxins. (23).

In addition to sneezing other risk factors include eating food prepared by a person with a skin infection that contain Staphylococcus aureus bacteria; eating food kept at room temperature; eating improperly prepared (cooked) food; and eating the same food as an individual who has symptoms. (21). The symptoms of Staphylococcus aureus food poisoning appear 1-6 hours after eating contaminated food. The symptoms lasts only 2 days or less and include, nausea, vomiting for up to 24 hours, diarrhea, loss of appetite, severe abdominal cramps, abdominal distention and mild fever. (21). A stool culture positive for Staphylococcus aureus is an effective diagnosis. Treatment for the disease is the use of the antibiotic penicillin, but in most countries, penicillin resistance is common and the first line therapy is most commonly a penicillinase resistant penicillin, for example, oxacillin or flucloxacillin. A combination therapy with gentamicin can sometimes be used. (24: 25). Full recovery from Staphylococcus aureus food poisoning usually occurs after 24-48 hours with treatment. (21).

Campylobacter

Campylobacteriosis is an infection by one of several species of Campylobacter, particularly Campylobacter jejuni. (26). Campylobacter jejuni is one of the major causes of bacterial gastroenteritis in the United States and worldwide. (26). Campylobacter jejuni has become recognized as a common bacterial cause of diarrhea. (27). Humans can become infected by eating undercooked chicken, unpasteurized milk and contaminated water. (6: 26). The infection occurs at any age and infants have an especially high rate of campylobacteriosis because of their immature immune defenses. (26; 27). Most healthy individuals probably develop some degree of immunity against Campylobacter jejuni as they mature. (26). This may be the result of a lower number of cases of Campylobacter infection in middle age and older persons. (26).

The incubation period for Campylobacter jejuni infection is one to seven days. (6: 26:27). The symptoms of Campylobacter jejuni infection includes a 12 to 48 hour period of fever, headache, muscle aches, malaise, bloody diarrhea, cramps and abdominal

pain. (6: 26: 27). Some individuals who have a Campylobacter jejuni have mild symptoms such as a few loose stools per day. (26). The disease normally lasts 7-10 days with antibiotic treatment. (26: 6: 27). Campylobacter jejuni infection can be treated with antibiotics such as erythromycin, azithromycin, levofloxacin and ciprofloxacin. (26:27). For most individuals a full recovery is expected in 7-10 days, but for others complications such as the Gillian-Barre Syndrome and reactive arthritis occur. (26: 6). When the Gillian-Barre Syndrome occurs Campylobacter jejuni triggers the immune system to attack nerves causing weakness and sometimes paralysis. (26).

Listeria monocytogens

Listeria monocytogens is the bacterium that causes the infection listeriosis. It is a facultative anaerobic bacterium, capable of surviving in an oxygen environment. Listeria monocytogens can grow and reproduce inside the host's cell and is one of the most virulent foodborne pathogens, with 20% to 30% of clinical infections resulting in death. (28).

Listeria monocytogens infection is caused by eating deli meats, processed meats, soft cheeses, vegetables, smoked seafood, and drinking unpasteurized milk , and the organism is cold and heat tolerant. (6: 29). The invasive infection by Listeria monocytogens causes the disease listeriosis. When the infection is not invasive any illness as a result of the infection is termed febrile gastroenteritis. (30). The manifestations of listeriosis include septicemia, meningitis (meningoencephalitis), encephalitis, corneal ulcer, pneumonia, and intrauterine or cervical infections in pregnant women, which may result in spontaneous abortion or still birth. (31: 32:33;34;29). Surviving neonates of fetomaternal listeriosis may suffer granulomatgosis infantiseptica-pyogenic granulomas distributed over the whole body- and may suffer from physical retardation. Influenza like symptoms, including persistent fever, usually precede the onset of the aforementioned disorders. (29). The incubation period for Listeria monocytogens infection is one to two days and the gastrointestinal symptoms such as nausea, vomiting and diarrhea may precede more serious forms of listeriosis. (29:6). The initial symptoms are often fever and muscle aches. (29). If diagnosed early enough antibiotic treatment of pregnant women or immunocompromised individuals can prevent serious consequences of the disease. (29). The antibiotics that are

known to be effective against Listeria monocytogens include ampicillin, vancomycin, ciprofloxacin, linezolid, azithromycin and penicillin. (29).

Pregnant women, older adults, and individuals with weakened immune systems are at higher risk for listeriosis. (29). The Center for Disease Control recommends the following measures for such individuals.

1. Do not eat hot dogs and luncheon meats unless they are reheated until steaming hot.
2. Avoid cross-contaminating other foods, utensils, and food preparation surfaces with hot dog packages, and wash hands after handling hot dogs.
3. Do not eat soft cheeses such as feta, brie and camembert cheeses, blue-veined cheeses, and Mexican style cheeses such as "queso blanco fresco". Cheeses that may be eaten include hard cheeses, semi-soft cheeses such as mozzarella, pasteurized processed cheeses such slices and spreads, cream cheese and cottage cheese.
4. Do not eat refrigerated pates or meat spreads. Canned or shelf-stable pates and meat spreads may be eaten.
5. Do not eat refrigerated smoked seafood, unless it is contained in a cooked dish, such as a casserole. Canned or shelf-stable smoked seafood may be eaten.
6. Do not drink raw (unpasteurized milk) or eat foods that contain unpasteurized milk.

Salmonella

Salmonellosis is an infection caused by salmonella bacteria.(6). The species Salmonella enterica and its many subspecies is largely associated with infections in humans. (35). Salmonella food poisoning is the most common cause of food borne illness. (36). A subspecies of Salmonella enterica serotype typhi cause salmonellosis that can lead to typhoid fever. (37). Salmonella enterica infection is usually contracted from foods such as poultry, pork, beef, and seafood, if the meat is prepared incorrectly or is infected with bacteria after preparation, infected eggs, egg products, and milk when not prepared, handle or refridgerated properly and tainted fruits and vegetables. (6: 36: 35).

The incubation period for salmonella infection is usually 1-2 days and has symptoms such as fever, diarrhea, vomiting, nausea, and abdominal pain (cramps) 12-72 hours after infection. (6: 36).

Salmonella food poisoning is more likely to occur and cause serious complications in individuals with weakened immune systems due to such conditions as HIV/AIDS. Cancer, diabetes, or taking steroid medications or undergoing chemotherapy. (36). Other individuals at risk include older adults, infants, children and pregnant women. (36). Salmonella food poisoning can result in serious complications that include dehydration, Reiter's syndrome, and a chronic condition, reactive arthritis. (6: 36: 38). Salmonella food poisoning is spread by eating or drinking food or beverages that have been contaminated with faeces that contain salmonella bacteria. (36).

Salmonella food poisoning is treated with a wide range of antibiotics. The Food and Drug Administration suggests guidelines to prevent salmonellosis such as cooking food to 145-160 F (thoroughly cooking), and liquids such as soups and gravies must be boiled. (39).

Shigella

Shigellosis is an infectious disease caused by a group of bacteria known as Shigella. (40). Shigella causes foodborne disease in primates, but not other mammals. (41). Shigella is one of the leading bacterial causes of diarrhea worldwide. (42). They are four subgroups of Shigella that cause disease to exist, namely, Shigella dysenteriae (subgroup A), Shigella flexneri (subgroup B), Shigella boydii (subgroup C), and Shigella sonnei (subgroup D). (42). Shigella foodborne infection is associated with fruits, vegetables and shell fish that have been contaminated with fecal matter, that is, transmission is by the fecal-oral route. (6: 42).

The incubation period of Shigella is 1-2 days and the symptoms include severe diarrhea with or without blood, fever, nausea, vomiting, abdominal cramps and painful bowel movements. (6: 42: 43). Shigella can also cause dysentery. (6: 42). Further complications of Shigella infection are seizures, toxic megacolon, reactive arthritis, and hemolytic uremic syndrome. (6: 42: 44). Shigella infection is most common during the summer months and usually affects children two to four years old, but rarely infects infants younger than 6 months old. (43). In rare cases the disease can cause seizures in young children. (42). Shigella bacteria produce toxins that can attack the lining of the large intestines, causing swelling, ulcers on the intestinal wall, and bloody diarrhea. (43). Shigella infections are very contagious and can be prevented with good hand washing practices and

thoroughly cooking foods. (45: 43). The infection can last 5-7 days and is treated with antibiotics such as ampicillin and fluoroquinolones. (6: 43). Antibiotics shorten that duration of the infection. (43

Escherichia coli 0157:H7

Escherichia coli 0157:H7 is an enterohemorrhagic strain of the bacterium Escherichia coli and is a cause of foodborne disease. (46). Escherichia coli 0157:H7 causes about 73, 000 outbreaks in the U.S.A annually. (47). It is also known to occur in Great Britain and Australia. (48: 49). Escherichia coli 0157:H7 foodborne infection has been associated with individuals eating alfalfa sprouts, ground beef (hamburgers, salami), ground pork, unpasteurized milk and juice, lettuce and drinking contaminated water. (6: 47).

The Escherichia coli 0157H7 infection is transmitted through the fecal-oral route, and most illnesses have been through the use of undercooked contaminated ground beef or ground pork being eaten. (47). The bacterium is readily destroyed by heat. The symptoms of Escherichia coli 0157: H7 are hemorrhagic diarrhea, and abdominal pain. (6: 50). The disease is treated with antibiotics and most victims can recover without treatment in five to ten days. The disease can be prevented by proper hand washing methods while preparing foods and after using the toilet.

Clostridium botulinum

Botulism is a rare but serious paralytic illness cause by a nerve toxin which is produced by the bacterium Clostridium botulinum. (51). There are three major kinds of botulism; food borne botulism, wound botulism and infant botulism. (51). Food botulism is caused by eating foods that contain the botulism toxin. Wound botulism is caused by toxin produced from a wound infected with Clostridium botulinum, and infant botulism which is caused by consuming the spores of the Clostridium botulinum bacteria, which then grow in the intestines and release toxins. (51). All forms of botulism can be fatal and are considered medical emergencies. (510. Foodborne botulism can be dangerous because many individuals can be poisoned by eating contaminated food. (51). The Clostridium botulinum bacterium is commonly found in the soil.

Clostridium botulinum food infection is normally associated with home-canned foods, such as asparagus, green beans, beets, corn, fish, tomatoes, chopped garlic in oil, chili peppers, and the

ingestion of uncooked foods in which contaminated spores have germinated and have elaborate toxin. (52: 51). The symptoms of Clostridium botulinum food infection are nausea, vomiting, diarrhea, cramps, blurred vision, drooping eyelids, slurred speech, difficulty swallowing, dry mouth, and muscle weakness. (6: 51). In food borne botulism the symptoms generally begin 18 to 36 hours after eating a contaminated food, but they can occur as early as 6 hours or as late as 10 days. (51). Twelve to seventy two hours after exposure paralysis illness can become present and lead to respiratory and musculoskeletal paralysis. (6). Clostridium botulinum food infection is treated by inducing vomiting and giving an antitoxin. (51)

Clostridium perfringens

Clostridium perfringens formally known as Clostridium welchii or Bacillus welchii is a gram positive, rod shape, anaerobic, spore forming bacterium of the genus Clostridium. (53). In the United kingdom and the United States of America clostridium perfringens bacteria are the third most common form of foodborne illness. (54). Some strains of Clostridium perfringens produce toxin in the intestine that cause illness. (55). It is estimated that Clostridium perfringens causes nearly one million cases of foodborne illnesses each year. (55).

Clostridium perfringens foodborne illness can be caused by eating beef, poultry, gravies, and dried or precooked foods. (54: 55). Although Clostridium perfringens may live normally in the human intestine, illness is caused by eating food contaminated with large numbers of Clostridium perfringens bacteria that produce enough toxin in the intestine to cause illness. (55). The incubation period for Clostridium perfringens food borne infection is 6-24 hours and the symptoms include diarrhea and abdominal cramp without fever or vomiting. (56). The duration of the illness is 24 hours or less, and in severe cases symptoms may last 1-2 weeks. (56). Complications include dehydration. Older adults, infants, and young children are individuals mostly at risk of getting the disease. (56). Clostridium perfringens infection is treated with oral rehydration, or in severe cases, intravenous fluids and electrolyte replacement can be used to prevent or treat dehydration. (56) Antibiotics are not recommended. (56).

According to the United States Department of Health and Human Services (2014) Clostridium perfringens food infection can be prevented by:

1. Thoroughly cook foods, particularly meat, poultry, and gravies.
2. Use a food thermometer.
3. Keep food hot after cooking. (at 140F or above0.
4. Microwave reheated food thoroughly (to 165For above)
5. Refrigerate perishable foods within two hours (at 40F or below).
6. Divide left overs into shallow containers and refrigerate immediately (Do not let them cool on the counter.

Bacillus cereus

Bacillus cereus is an aerobic, spore forming bacterium that is commonly found in soil. (58). The Bacillus cereus bacteria produce toxins. (56). The Bacillus cereus bacterial food poisoning is usually associated with raw vegetables and processed foods, cooked meat and vegetables, boiled or fried rice, vanilla sauce, custards, soups, and raw vegetable sprouts. (58: 56).

Two types of illnesses have been attributed to the consumption of food contaminated with Bacillus cereus bacteria. (58: 56). The first type is diarrheal and is characterized by non-bloody diarrhea and abdominal pain. It has an incubation period of4-26 hours after the ingestion of food, with symptoms that last 12-24 hours. (58:56). The second type of illness with is known as the emetic type, is characterized by an acute attack of vomiting, and occurs within 1-5 hours after the consumption of contaminated food. (58:56). Diarrhea is not a common feature of this type of illness. (59).

Bacillus cereus food poisoning is usually self-limiting and only symptomatic treatment is given. (56:60). Patients are administered electrolyte containing fluids and serious cases are admitted to hospital and given intravenous fluids if severe dehydration develop. (56; 60). Bacillus cereus foodborne illness is likely to be under reported because of its relatively mild symptoms, which are of a short duration. (61). Bacillus cereus food poisoning can be prevented by keeping hot foods hot (over 140F), and cold foods cold (below 40 F). (56). Foods should be stored in a wide, shallow container and refrigerated as soon as possible. (56).

Brucella

Brucella are small, gram negative, non-motile, spore forming, rod shaped (coccobacilli) bacteria. (62). The foodborne disease

caused by Brucella bacteria is called Brucellosis, and is also known as Bang's disease, Crimean fever, Gibraltar fever, Malta fever, Rock fever, or undulant fever. (63:64). Brucellosis is known to occur in the United States of America, Canada, Europe, Republic of Ireland, New Zealand and Australia. (65:66:67: 68:69).

Brucellosis is caused by the ingestion of unpasteurized milk or meat from infected animals, or close contact with their secretions. (70). The disease can be transmitted from human to human through sexual contact, or from mother to child, which is rare but possible. (71). The symptoms of Brucellosis are sweating, fever, joint and muscle pain. (72). The disease is treated with antibiotics such as doxycycline and rifampin in combination for a period of 6-8 weeks. The diagnosis for the disease involves tests for the Brucella bacteria in blood, bone marrow or other body fluid. (72). About 2% of all Brucellosis cases result in death. (72). The disease can be prevented by using only pasteurized milk and using meat that past all inspection standards.

Chapter 2

Viruses in Foodborne Disease

In the recent years, there has been an increase in the incidence of food-borne disease world -wide with viruses now recognized as a major cause of these illnesses. (73:74). The viruses implicated in food-borne disease are the enteric viruses, which are found in the human intestines, excreted in human faeces, and transmitted by the fecal-oral route. (73). A lot of different viruses are found in the human gut, but not all are recognized as food-borne pathogens. (73). The enteric viral pathogens present in human feces include noroviruses (Norwalk like viruses), enteroviruses, adenoviruses, Hepatitis A virus (HAV), Hepatitis E virus (HEV), rotaviruses, and astroviruses, most of which have been associated with food-borne disease outbreaks. (73). The noroviruses are the major group identified in food-borne outbreaks of gastroenteritis, but other human derived and possibly animal derived viruses can possibly be transmitted via food. (73).

The diseases caused by enteric viruses fall into three main categories: gastroenteritis, enterically transmitted hepatitis, and illnesses that can affect other parts of the body such as the eye, the respiratory system, and the central nervous system including conjunctivitis, poliomyelitis, meningitis and encephalitis. (73). Five enteric viruses-noroviruses, HAV, HEV, rotaviruses, and astroviruses are reported to cause food-borne disease outbreaks world-wide. (75:76). The transmission of a virus is dependent not only with its interaction with the host, but on its interaction with the environment outside the host. (77). A robust survival of enteroviruses exists on fomites, in waters, soil and food. (77). This high survival rate of enteroviruses in foods could facilitate their involvement in food-borne diseases.

Noroviruses

Noroviruses, previously known as small round structured viruses (SRSVs) and Norwalk like viruses (NLVs), are now the most widely recognized viral agents associated with foodborne and water borne outbreaks of non bacterial gastroenteritis and probably the most common cause of food borne disease world-wide. (73: 74). Norovirus food borne illnesses occur all year round, and cause illness to individuals of all ages. (73: 74). Noroviruses

have been associated with a broad range of food items. (74). Food borne Norovirus outbreaks resulting from preharvest contamination of foods such as shell fish and post harvest contamination through food handling have been reported world-wide. (73). Among these are several outbreaks resulting from the consumption of Norovirus contaminated shell fish, bakery products, delicatessen meats, sandwiches, raspberries, water and ice. (78:79:80:81:82:83:84:85:86). Presymptomatic infection among food handlers has also been shown to cause outbreaks of food borne Norovirus infection. (87:88). Noroviruses are extremely infectious and cause epidemic gastroenteritis. (73). The infectious dose is believe to be low 10-100 virus particles. (89).

Determination of the original source of norovirus is often problematic because several modes of transmission frequently operate during norovirus gastroenteritis outbreaks. (73). Although the initial route of transmission may be through the consumption of contaminated foods, secondary transmission via direct contamination of the environment or person to person contact also occur. (73). This can result in wide dissemination whereby the infection quickly spreads through institutions, schools, camps, resorts, and cruise ships and cause large scale epidemics with more than 50% attack rates. (73). The illness overall is relatively mild, but more severe illness and death occur in at risk groups such as the elderly or people with underlying disease. (74).

Hepatitis A Virus (HAV)

Hepatitis A virus (HAV) is very stable, showing high resistance to chemical and physical agents such as drying, heat, low pH, and solvents and has been shown to survive in the environment, including seawater and marine sediments for more than three months. (90). HAV can be cultured in several different primate cell lines including African green monkey kidney cells, fetal rhesus monkey kidney cells (FRhK-4 and FRhK-6), and human fibroblasts (HF), but wild type strains are difficult to culture and generally do not produce CPE in cell cultures. (73). HAV has been associated with numerous outbreaks of food-borne disease. (73). Contamination generally occurs either preharvest or during food handling, and there are documented outbreaks of the disease resulting from consumption of HAV contaminated shell fish such as clams, oysters, and mussels. (73: 91: 92: 93: 94: 95). In the above mentioned outbreaks sewage was generally the source of

pollution. (73). Pre-harvest contamination of fruits and vegetables such as strawberries, raspberries, blueberries, lettuce, and green onions has also been reported, and has resulted in outbreaks of the disease in countries such as Findland and New Zealand where the populations have low or no immunity to the disease. (96:97: 98:99:100:101). The source of contamination of fruits and vegetables were reported to be either infected fruit pickers or contaminated irrigation waters. (73).

Another main source of HAV infection is from food handlers and food processors. (73). Because HAV can be shed before symptoms become apparent and more than one million infectious particles can be excreted per gram of feces. (73). HAV produce infected harvesters and food handlers without knowing, and they can become a source of contamination. (73). In areas of poor hygiene practices, food harvesting and food processing can present a risk to human health. (73). Food borne outbreaks of HAV are relatively uncommon in developing countries where there are high levels of immunity in the local population, but tourists in these regions can be susceptible if they are not vaccinated. (73).

HAV has an incubation period of two to six weeks with an average of 28 days. (73: 102). The initial (non-specific) symptoms of the HAV infection are fever, headache, fatigue, appetite loss, dark urine, light stools, nausea and vomiting with occasional diarrhea. (73:103). One to two weeks after HAV infection characteristic symptoms of hepatitis appear such as viremia and jaundice. (73). Diagnosis is based on the detection of anti-HAV IgM antibody which can be detected before the onset of symptoms (and infections often occur in conditions of poor sanitation and overcrowding. (104). Hepatitis A can be prevented by vaccination, good hygiene and sanitation. (105).

Hepatitis E Virus (HEV)

HEV is long known as an endemic disease in areas with poor hygienic conditions, causing acute self-limiting hepatitis.(76). HEV is believed to be a major etiologic agent of enterically transmitted non-A, non-B hepatitis in humans world-wide. (106). The virus occurs widely in Asia, Northern Africa, and Latin America, including Mexico, where water borne outbreaks are common. (73). It was originally believed that HEV did not occur in industrialized countries but in recent years it has been identified in Europe, Australasia, and the United States of America. (73). The virus rarely

cause overt disease in industrialized countries. (107: 106). The HEV virus has been isolated from sewage in Spain, France, Greece, Italy, Austria, and the United States of America. (108: 109).

Food borne outbreaks of HEV are most common in developing countries with inadequate environmental sanitation. (73). Large water borne outbreaks have also been reported in Asian countries. (110: 106). HEV was not thought to be endemic in developed countries, and the first reported human (non-travelled related) cases of acute hepatitis E in the United States of America occurred in 1997. (73). The HEV virus was identified in domestic swine and human in many countries including Argentina, Australia, Austria, Canada, Germany, Greece, Japan, Korea, The Netherlands, New Zealand, Spain and Taiwan. (111: 112: 113).

Recent reports from Japan showed that the HEV virus may be transmitted to humans by close contact with infected swine or from the consumption of contaminated raw or undercooked pork, wild boar liver, and deer meat. (114:115). Similarly, fecal contamination of run off waters from pig farms or from lands on which untreated pig manure has been spread could contaminate irrigation and surface waters with subsequent HEV contamination of fruits, vegetables, and shell fish. (73). HEV is shed in the feces and bile of swine for 3-5 weeks after infection. (116). Although there is increasing evidence of the zoonotic transmission of HEV, the risk factors are still largely unknown. (73).

The transmission of the virus is through the fecal-oral route. (73). The virus infects the liver and produces symptoms of hepatitis after a 22-60 day incubation period. (73). The symptoms include viremia, nausea, dark urine, and general malaise. (73). The virus is excreted in the bile and feces of infected humans from 2 weeks before the elevation of liver enzymes and continues until the enzyme levels return to normal. (73). The infection is mild and normally self- limiting. Identification and diagnosis is generally by detection of IgM and IgG responses in patients' sera to recombinant HEV protein antigens or by molecular assays to identify the virus in feces and sera. (73). Generally, the mortality rate from hepatitis E infections is about 1% but may reach as high as 17-20 % in pregnant women. (110:106). The major mode of transmission appears to be contaminated water and secondary person to person transmission which is estimated to be 0.7-8.0 %

and is relatively uncommon. (73:110). A preventative vaccine (HEV 239) has been approved for use in China. (117).

Rotaviruses

Rotaviruses are classified in the genus Rotavirus and the family Rotaviridae, a large family composed of nine genera. (73). There are five species of rotavirus, designated Rotavirus A (simian rotavirus) through Rotavirus E (porcine rotavirus). Two possible species, Rotavirus F (avian rotavirus) and Rotavirus G (avian rotavirus). Most human infections are caused by Rotavirus A, B, and C, but all rotaviral species can infect a range of vertebrates, including primates, ruminants, rodents, and birds. (118:119). Although many rotaviruses can be grown in cell cultures, they proved difficult to cultivate in vitro, and growth is restricted to a few cell lines derived from monkey kidneys. (73).

Rotaviruses are the major cause of severe diarrhea and gastroenteritis in infants and young children. (73). It is estimated that rotaviruses cause more than 130 million cases of diarrhea in children under 5 years of age annually world-wide. (120). Rotaviral infection is a particularly serious problem in developing countries where up to 600,000 deaths occur annually among children. (73). In the United States of America rotaviruses are estimated to cause about 4 million infections per year resulting in almost 70,000 hospitalizations and more than 100 deaths annually. (121:119). Although the disease occurs in all age groups, it is considered to be a mild infection in adults, hence the true extent of adult infections is not known. (73).

Although the animal and human strains are usually distinct, some human strains are closely related to animal strains and cross species infections do occur. (119). The rotavirus infection is not generally recognized as food-borne, but outbreaks associated with food and water have been reported in a number of countries. (119).The virus is stable in the environment, hence infection can occur through consumption of contaminated water or food and contact with contaminated surfaces. (73). Eleven food borne outbreaks consisting of 460 cases of rotaviral gastroenteritis were reported in New York between 1985 and 1990. (73). Seven outbreaks were associated with food service premises and the implicated foods included salads, cold foods, shepherd's pie, and water or ice.(119). A recent study conducted in the Netherlands,

the lack of food handling hygiene was identified as one of the main risk factors for rotavirus infection. (122).

Large scale outbreaks of rotaviral gastroenteritis have been reported in Japanese primary schools with more than 3,000 cases recorded for one outbreak. (123: 124). School lunches from a central facility were suspected as the vehicle of infection, but no rotavirus was isolated from food or water.(73). In Costa Rica, market lettuce was found to be contaminated with rotavirus and HAV at a time when there was a high incidence of rotaviral diarrhea in the community. (125). Water borne rotaviral outbreaks have been reported in many countries, including China, Germany, Isreal, Sweden, Russia, and the United States of America. (126: 119). Large numbers of rotaviral particles are excreted in feces after infection, and calves infected with rotavirus are known to shed 10,000,000,000 particles per gram of feces. (73). Contamination of water supplies by animals could therefore be a source of water borne disease. (73). Links have been reported between human and animal rotaviral disease, and it is possible that zoonotic transmission of rotavirus may also occur. (73).

The incubation period for rotavirus infection is 1-2 days.(73). The characteristic symptoms of vomiting and watery diarrhea develop quickly and persist for 3-8 days, frequently accompanied by fever and abdominal pain. (73). Dehydration is a key factor that contributes to a high infant death rate from s available. The virus is shed in feces for 5-7 days. The main route of transmission of the rotavirus is fecal-oral. Because rotaviruses most often infect young children, the major route of transmission is thought to be person to person through care-givers and the general adult population. (73). Rotavirus can also infect adults and have also been associated with food and water borne outbreaks. (73). Rotavirus B strains have caused large epidemics in human adults in China, and group C rotavirus causes sporadic outbreaks in children. (120: 119). Rotavirus disease is more common during the winter months in countries with a temperate climate. (73). In tropical regions, outbreaks can occur both in cooler and drier months and throughout the year especially where transmission is related to contaminated water supplies and where no sewage treatment system exist. (126:127).

Some immunity develops after infections although it does not give complete protection from future infection. (73). However,

repeat infections are often less severe than the original infection. (73). An oral rotavirus vaccine was developed in the late 1980's, but the distribution was delayed after lengthy investigations into possible complications associated with the vaccine. The vaccine is now approved for commercial global distribution. (73). Chelating agents such as EDTA disrupt the outer shell and inactivate rotaviruses. (73). Treatment with disinfectants such as chlorine, phenol, formalin, and 95% ethanol is also effective against rotaviruses. (128). Normal cooking temperatures are usually sufficient to inactivate rotaviruses. (73). Rotaviruses have been found in water and sewage that are resistant to the chlorine levels present in drinking water and are persistent in the environment. Human , and in rotavirus can survive for several weeks in river water at 4 degrees Centigrade and 20 degrees Centigrade. (73).

Astroviruses

Astroviruses were first recognized in 1975 and named according to their star-like appearance under the electron microscope. (129). Astroviruses belong to the family Astroviridae, and human astroviruses is the single type species in the genus Mamastrovirus. (73). Astroviruses are distributed world-wide and in addition to man the viruses have been isolated from birds, cats, dogs, pigs, and cows.(73). Although human, bovine, feline and porcine astroviruses have been isolated in primary embryonic kidney cell lines such as human embryonic kidney cell (HEK), only human and porcine astroviruses have been adapted to grow in established cell lines and trypsin is required in the growth medium to boost infectivity. (73). The human strains are all antigenically distinct from the bovine and ovine strains. (73). A second genus of astrovirus which contains two serotypes infect birds, including turkeys and ducks. (118).

Epidemiological evidence of transmission by food is currently limited, but infections in shell fish and water have been reported. (130: 131). An outbreak of astrovirus infection occurred in Japan in 1991 and the outbreak was traced to lunches prepared by a common supplier. (130). There are several Japanese reports of astrovirus genomes identified in shell fish, and there is evidence that astroviruses appear to contribute to food borne outbreaks of gastroenteritis mainly through the consumption of contaminated oysters. (132).

The main feature of astrovirus infection in both humans and animals is a self- limiting gastroenteritis, with the majority of infected cases detected in young children under one year of age. (133 : 131). A surveillance study in the United Kingdom reported that astroviruses were the most common cause of infectious gastrointestinal disease. (134). Although astroviruses cause a mild infection in adults, they are associated with gastroenteritis in immune-compromised adults. (73). The incubation period for astrovirus infection is 3-4 days and the symptoms include diarrhea, fever, nausea, and general malaise with occasional vomiting. (73). Normally, diarrhea persists for only 2-3 days but can be prolonged for up to 14 days with virus excretion in the feces. (73).

Transmission of astrovirus is through the fecal-oral route via food, water and person to person contact. (73). Symptomatic excretion of the virus occurs in feces of 5-20% of neonates and young children and is a significant source of infection, especially in nurseries, child care centers, and hospitals. (135: 131: 133). In temperate climates, a seasonal peak in winter and spring occurs, but infections may occur throughout the year. (73). The detection of astrovirus is carried out mainly by Electron Microscopy of stool specimens, molecular assays, or by combined culture-PCR methods. (73).

Astrovirses are resistant to extreme environmental conditions and have a heat tolerance that allow them to survive up to 50 degrees centigrade for one hour. (73). The virus is stable in acid conditions (pH 3.0), and is resistant to chemicals including Chloroform, lipid solvents, and alcohols and to non-ionic, anionic, and zwitterionic detergents. (131).

Chapter 3

Fungi in Foodborne Disease

Fungi are a group of eukaryotic organisms that include microorganisms of the genus Fusarium, penicillin, Aspergillus, Claviceps, and mushrooms. Fusarium, Penicillin, Aspergillus and Claviceps grow on the normal flora of food-fruits and vegetables-and can cause infection in normal and immuno-compromised individuals if contaminated food is ingested. Fungal food poisoning is still in the research stage and it is not enough to understand that there is a known fungal toxin and several species of fungi can produce the same toxin with similar symptoms. (136). Mushrooms are normally eaten by man, but some species of mushrooms are poisonous and can cause infection that leads to death. Pencillium, Fusarium and Aspergillus are also used by man for their beneficial effects.

Fusarium

Fusarium is a large genus of filamentous fungi normally found in soil and the flora of plants-fruits and vegetables. Some species of Fusarium produce mycotoxins in cereal crops that can affect human and animal health if they are ingested. More than 100 species of Fusarium have been identified, but only a few species cause infections in humans. (137). Fusarium solani is the most frequent cause of invasive disease (in half of all cases), followed by F. oxysporum, F. verticilloides and F. proliferatum.

In humans with normal immune systems, fusarial infections may occur in the nails (onychromycosis), and in the cornea (keratomycosis or mycotic keratitis). (138). In humans whose immune systems are weakened in a particular way, neutropenia (very low neutrophil count), aggressive fusarial infections penetrating the entire body and blood stream may be caused by members of the F. solani complex, F. oxysporum, F. verticilloids, F. proliferatum and rarely other fusarial species. (139). Mass casualties occurred in the Soviet Union in the 1930s and 1940s when Fusarium contaminated wheat flour was baked into bread, causing alimentary toxic aleukia with a 60% mortality rate. The symptoms began with abdominal pain, diarrhea, vomiting and prostration, and within days, fever, chills, myalgias, and bone marrow depression with granulocytopenia and secondary sepsis.

Further symptoms included pharyngeal or laryngeal ulceration and diffuse bleeding into the skin, melena, bloody diarrhea, hematuria, hematemesis, epistaxis, vaginal bleeding, pancytopenia and gastrointestinal ulceration. Fusarium sporotrichoides contamination was found in affected grain in 1932, initiating research for medical purposes and for possible use in biological warfare.

Fusarium venenatum is produced industrially for use as human food by Marlow Foods, Ltd., and is marketed under the name Quorn in Europe and North America. Some consumers of Fusarium products have shown food allergies similar in nature to peanut and other food allergies. Individuals with known sensitivities to molds should exercise caution when consuming such products. (140).

Claviceps

The genus Claviceps contains a group of fungi no1rmally referred to as Ergot or Ergot fungi. (141). The most prominent member of the group of about 50 known species is Claviceps purpurea. Claviceps fungi grow on rye and related plants. Economically significant species include Claviceps purpurea (parasitic on grasses and cereal), Claviceps fusimormis (parasitic on pearl mille and buffel grass), Claviceps pasipali (parasitic on dallis grass), and Claviceps Africana (parasitic on sorghum). (142). Claviceps purpurea most commonly affects out crossing species such as rye (its most common host), as well as triticale, wheat, and barley, and it rarely affects oats.

While growing on plants, Claviceps purpurea produces alkaloids that can cause ergotism in humans who consume grains contaminated with a fruiting structure called an ergot sclerotium. (143). Ergotism is the name given to the food poisoning caused when individuals consume plant material containing the ergot alkaloid, such as ergot contaminated grains. Human poisoning due to the consumption of rye bread made from ergot infected grain was common in Europe in the middle ages. (144). The neurotropic activities of ergot alkaloids may cause hallucinations and attendant irrational behavior, convulsions, and even death. (145: 146). Other symptoms include strong uterine contractions, nausea, seizures, and unconsciousness. The common name for ergotism is “St. Anthony’s fire” in reference to the order of monks and the severe burning sensations in the limbs which is a known symptom.(147).

The burning sensations in the limbs are caused by effects of ergot alkaloids on the vascular system due to vasoconstriction, sometimes leading to gangrene and the loss of limbs due to severely restricted blood circulation. The monks of the Brothers of St. Anthony treated egotism with balms containing tranquilizing and circulation stimulating plant extracts. (148).

Aspergillus

Aspergillus is a genus of deuteromycetes fungi consisting of a hundred or more mold species found in various climatic environments world-wide. Some Aspergillus species cause serious disease in humans and animals. The most common pathogenic species are Aspergillus fumigatus, Aspergillus flavus, and Aspergillus parasiticus which produces aflatoxin. Aflatoxins are both acutely and chronically toxic to man and other animals. (149). Aflatoxins are known carcinogens.

Aspergillus fumigatus and Aspergillus flavus contaminate foods such as nuts and oilseeds. Aspergillus ochraceus is widely distributed in foods such as beans, dried fruit, biltong, salt fish, peanuts, pecans, betel nuts, barley, wheat, flour and rice. (149). Aspergillus orchraceus produces Ochratoxin A. Food borne illnesses caused by Aspergillus species are due to the consumption of contaminated food by humans and other animals. The toxins produce by these species cause the symptoms and illness.

Penicillium

Penicillium is a genus of ascomycetous fungi of importance in the natural environment as well as food and drug production. Penicillium is best known for antibiotic production, for example, the species Penicillium chrysogenum. Some species of Penicillium produce highly toxic mycotoxins, for example, Penicillium verrucosum, which produces Ochratoxin A, Penicillium viridicatum which produces Ochratoxin. Penicillium species grow on wheat, barley, apples, pears, garlic, citrus fruits, and stored food such as bread. (150). Species of Penicillium known to be pathogenic to animals including man are P. corylophilum, P. fellutanum, P. implicatum, P. janthinellum and P. viridicatum. The food illness caused by Penicillium species is associated with the ingestion of contaminated food.

Symptoms of Fungal Food Poisoning

When fungal food poisoning occur the symptoms are normally similar. Generally there is an acute fungal food poisoning

incubation period which is short, with first gastrointestinal symptoms such as abdominal discomfort, nausea, vomiting, abdominal distention, loss of appetite, and occasional diarrhea. Depending on the type of mycotoxin there is the occurrence of damage to the liver, kidney, nervous system and blood system. Some patients experience symptoms such as an enlarged liver, tenderness, abnormal liver function and jaundice (common in Aspergillus flavus and Penicillium poisoning), proteinuria, hematuria, or oliguria anuria (Penicillium poisoning prone). Some Penicillium fungi toxins cause neutropenia or lack of thrombocytopenia bleeding. Some Aspergillus species produce poisoning prone to the nervous system symptoms, dizziness, headache, retardation, agitation, ataxia, convulsion, coma and paralysis. Many patients die of liver failure, kidney failure, or central nervous system paralysis, and the fatality rate can be as high as 40-70%. Some species of fungi cause cancer.

Chapter 5

Mycotoxins

In metabolites of biological origin. Despite efforts to control fungal contamination, toxigenic fungi are ubiquitous in nature and occur regularly in world-wide food supplies due to mold infestation of susceptible agricultural products such as cereals, grains, nuts, and fruits. (151). Thousands of mycotoxins exist, but only a few present significant safety challenges. (151).

Mycotoxins can enter the food chain as a result of fungal infection of crops, either by being directly by humans, or being used in livestock feed. Mycotoxins greatly resist decomposition or being broken down in digestion, so they remain in the food chain in meat and poultry products. Temperature treatments such as cooking and freezing do not destroy some mycotoxins.

There are six classes of mycotoxins that are of importance to man both medically and agriculturally. These classes cause disease in humans and animals and are categorized as:

1. Aflatoxins
2. Ochratoxin
3. Citrinin
4. Ergot Alkaloids
5. Patulin
6. Fusarium Toxins

Aflatoxins

There are at least 14 different types of aflatoxins produced in nature. (152). Of these 5 aflatoxins and their metabolites are of medical importance. Aflatoxin B1 and B2 are produced by Aspergillus flavus and Aspergillus parasiticus. Aflatoxin B1 is considered the most toxic. Aflatoxin G1 and G2 are produced by Aspergillus parasiticus. Aflatoxins M1 and M2 were first discovered in the milk of cows. That were fed on moldy grain. There are metabolites of Aflatoxins B1 and B2 respectively and are found in the milk of cattle and humans. Other aflatoxins are also found in Aspergillus spp., and the metabolite Q1 along with the other mentioned metabolites are formed by conversions in the liver. Aflatoxins are considered the most important mycotoxins and they are widely researched.

Aflatoxins have been isolated from all the major cereal crops, and from sources as diverse as peanut butter and cannabis. The staple commodities regularly contaminated with aflatoxins include cassava, chillis, corn, cotton seed, millet, peanuts, rice, sorghum, sun flower seeds, tree nuts, wheat, and a variety of spices intended for human consumption. (153:154). When processed aflatoxins get into the general food supply where they have been found in both pet and human foods, as well as feedstock for agricultural animals. Aflatoxin transformation products are sometimes found in eggs, milk, products and meat when animals are fed contaminated grain. (155).

Ochratoxin

Ochratoxin is a mycotoxin that comes in the three secondary metabolite form A, B and C. Ochratoxin A, B, and C are produced by Aspergillus and Penicillium species. The three forms differ in that Ochratoxin B is a non-chlorinated form of Ochratoxin A, and Ochratoxin C is an ethyl ester form of Ochratoxin A. (156). Ochratoxin is considered to be nephrotoxic, teratogenic, and immunotoxic, and has been classified as a 2B carcinogen-probable human carcinogen. (151). Ochratoxin A is the main toxin in the group and has been labeled as a carcinogen, and has been linked to tumors in the human urinary tract, although research in humans is limited by confounding factors. (156:157).

Ochratoxin A is found in wheat, corn, and oats having fungal infection and in cheese and meat products of animals consuming Ochratoxin contaminated grain. (158). Aspergillus ochraceus, a producer of Ochratoxin A is found on dry foods such as dried and smoked fish, soybeans, garbanzo beans, nuts and dried fruit. Aspergillus carbonarius is the major pathogen in grapes and grape products including raisins, wine and wine vinegars. (159).

Although reported to occur in foods around the world, the major regions of concern are Europe and for some food Africa. (151). The Joint committee on Food Additives of the Food and Agricultural Organization and the World Health Organization presented data indicating that cereals, wine, grape juice, coffee and pork are the major sources of Ochratoxin exposure at levels of 58%, 21%, 70%, 5%, and 3% of total Ochratoxin intake respectively. The levels reported range from 100 to 700 ng/kg in cereals, 30 to 9000 ng/L in European wines, 170 to 1300 ng/kg in coffee, and 150 to 2900 ng/kg in pork. (160). The presence of Ochratoxin in

European wines is a relatively recent concern, with red wines typically containing higher levels of Ochratoxin than rose' or white wine. (160).

Citrinin

Citrinin is a mycotoxin that was first isolated from Penicillium citrinum, but has been identified in over a dozen species of Penicillium and several species of Aspergillus. Some of these species are used to produce human foodstuffs such as cheese (Penicillium camemberti), sake, miso, and soy sauce (aspergillus oryzae). Citrinin is associated with yellow rice disease in Japan and acts as a nephrotoxin in animal species tested. (161). Although Citrinin is associated with human foods such as wheat, rice, corn, barley, oats, rye, and food colored with Monascus pigment its full significance in human health is unknown. Citrinin can also act synergistically with Ochratoxin A to depress RNA synthesis in murine kidneys. (161).

Ergot Alkaloids

Ergot alkaloids are compounds produced as a toxic mixture of alkaloids in the sclerotia of species of Claviceps, which are common pathogens of various grass species such as wheat and rye. The ergot alkaloids can be classified into two classes, (a) derrivatives of 6, 8-dimethylergoline, and (b) lysergic acid derrivatives. (162). The ingestion of ergot sclerotia from infected cereals, commonly in the form of bread produced from contaminated flour, cause ergotism, the human disease historically known as St. Anthony's fire.

Ergotism is the oldest identified mycotoxicosis in humans. The Sparatans apparently suffered an ergot epidemic in 430 BC and European epidemics as far as 857 A.D (163). Ergotism has also been associated with the Salem witch trials in the 1600s in Massachusetts. (164). More recent outbreaks, associated with economic upheaval and war, have occurred in Russia (1924 and 1944), Ireland (1929), France (1953), and Ethiopia (1978). (151). There are two forms of ergotism: gangrenous, affecting blood supply to extremities, and convulsive, affecting the central nervous system. Modern methods of grain cleaning (processing) have significantly reduce ergotism in humans but ergot poisoning continues to pose a challenge for the livestock industry.

Patulin is a mycotoxin produced by Penicllium expansum , Aspergillus paecilomyces and other Penicillium spp. Apricots, grapes, peaches, pears, olives, cereals, apples, figs, and low acid

fruits are considered sources of Patulin. (165). Patulin is not found in intact fruit because it is damage to the surface of the fruit that makes it vulnerable to Penicillium infection. (166). Therefore a critical point for controlling fruit quality is the point at which the fruit enters the processing line.

Recent studies have determined that Patulin is not a carcinogen although it has been reported to damage the immune system in animals. (167:168). Historically apple juice has been the product of great concern with regard to Putalin contamination. (151). Many of the investigations on Patulin stability during food processing have been focused on fruit juices, particularly apple juice. In 2004, the European community set limits to the concentration of Patulin in food products. They currently stand at 50ug/kg in all fruit juice concentrations, at 25 ug/kg in solid apple products used for direct consumption, and at 10 ug/kg for children's apple products, including apple juice. (168: 169).

Filtration to clarify juices and concentrates reduce Patulin levels up to 40%. (170). Patulin is destroyed by the fermentation process of apple juice and is not found in apple beverages such as cider. (171). Findings pertaining to the effect of heat on Patulin vary. No loss of Patulin in apple juice was reported by some researchers, while others found partial destruction using high temperature/short term pasteurization, or a 25% loss of natural contamination during pasteurization or evaporation conditions-70-100 degrees centigrade. (172: 173; 174). It is thought that the pH of apple juices lends heat stability to Patulin, accounting for the variability in the results. (151).

Fusarium Toxins

Fusarium toxins are mycotoxins produced by over 50 species of Fusarium and have a history of infecting cereals such as wheat and maize. (175: 176). Fusarium toxins include Zearalenone, which is not correlated to any fatal toxic effects in animals or humans; fumonisms, which affects the nervous systems of horses and may cause cancer in rodents, and trichothecenes, which are most strongly associated with chronic and fatal toxic effects in animals and humans. Other major types of Fusarium toxins include: beauvercins and euniatins, butenolide, equisetin, and fusarins. (177). From a medical and agricultural perspective, as well as, research progress the Fusarium toxins of major importance are trichothecenes, fumonisms, and Zearalenone.

Trichothecenes

There are approximately 180 trichothecenes known to exist but only a few are significant to human health. (151). The trichothecenes found in human foods are deoxynivalenol (DON), 3-ethyl deoxynivalenol, T-toxin, and nivalenol. Deoxynivalenol (DON) is the most prevalent of the trichothecenes in human foods. (151). The biosynthetic pathway for trichothecene production is known for some Fusarium species. (178). Eleven genes involved in the trichothecene pathway have been cloned. (179). These findings allow researchers to pursue traditional plant breeding and transgenic approaches to mycotoxin control. (151).

Although human DON exposure may be within the range of doses shown to be immunotoxic to rodents, human exposures and responses to the toxin are ill defined. (151). A small number of urine samples from humans in a high risk region (with greater wheat and corn food intake) had 37 ng DON/ml (14 to 94 ng/ml) range, whereas samples from a low risk region had 12ng DON/ml (4-18 ng/ml) range, a significant difference.(180). Several thousand individuals were affected by gastrointestinal distress in an incident in the Kashmir Valley of India in 1987. (151). Ninety-seven reported feelings of fullness and mild to moderate abdominal pain within 15 minutes to 1 hour after consuming their breakfast or evening snack consisting of locally produced or home- made wheat bread. Others reported symptoms that included throat irritation (63%), diarrhea (39%), vomiting (7%), blood in stools (5%), and facial rash (2%). Increase incidence of upper respiratory tract infections were reported in children who had consumed the wheat bread for more than a week. Illnesses subsided when the consumption of bread was ceased. Samples of flour and wheat in local markets contained DON (11/17 had toxin levels of 0.346 to 8.38 ug/g), nivalenol (2/19 had levels of 0.03 to 0.1ug/g), T-2 toxin (4/19 had levels of 0.55 to 1.0 ug/g), and 3-acetylDON (4/19 had levels of 0.6 to 2.4 ug/g), but were negative for aflatoxins and ergot alkaloids.(181). These results provided solid evidence for the potential health implications of fairly low exposure to the toxin and emphasized the need for more work to define human risk from this common food borne contaminant. (151). There is a need to define the role of DON in the impairment of the human immune system. (151).

Minimal tillage, nitrogen fertilizers, application azoxystrobin (fungicide), or glyphosate (herbicide), and the production of grains

where maize had been grown the previous year are the main risk factors associated with increase DON accumulation in food crops. (182). Current food processing techniques do not significantly contribute to DON remediation, either by reduction or detoxification in human or animal foods. (151). The combination of high pH (10.0) and high heat (100 degree centigrade for 60 minutes and 120 degrees centigrade for 30 minutes), and the treatment of DON in an aqueous buffer solution produce partial to complete destruction. (183). Treatment of wheat and corn with approved food additives such as dry and aqueous sulfite gas and ozone, has also been investigated. Aqueous sodium bisulfite had the greatest reductive effect by reaction with DON to produce acid-stable sulfonate adduct (DON-S); however, this adduct was hydrolyzed to DON under alkaline conditions. Upon bread baking, the toxin levels increase by 50 to 75% due to alkaline hydrolysis of the DON sulfonate adduct. (184). DON levels in corn were reduced by as much as 95% by autoclaving at 121 degrees centigrade with 8.33% aqueous sodium bisulfate. This corn was mixed with a basal diet and fed successfully to pigs, indicating that this treatment may be a viable option depending on the end use of the contaminated grain. (185).

Removing DON from feed barley by an abrasive dehulling (pearling) process was shown to significantly reduce toxin levels. Naturally contaminated (at various levels) barley was pearled for 15 seconds to consistently leave 34% of the initial concentration of the toxin and 85% of the grain mass. Longer treatment resulted in significant grain losses, but scaled-up remains to handle to commercial feasibility. (186). Grain milling portioned DON and Zearalenone to the various kernel fractions (bran>shorts>flour). Zearalenone was reduced below 1 ppm in all of the 27 flour samples; however, 3 of 27 flour samples contained DON levels greater than 1ppm. The Food and Drug Administration advisory information suggests that DON levels above 1ppm are not acceptable for use in products for human consumption. Reduction effectiveness depended upon the extent of penetration of the fungi into the kernel. (187). This limited the practical implications of this method to control DON levels in milled products. Other methods investigated without success include alkaline processing and high temperature (autoclaving) and high pressure (extrusion). (188: 189). Ultimately, continued vigilance is necessary to screen

potentially contaminated grains and properly dispose of lots with DON levels higher than allowed for specific purposes. (151).

Zearalenone

A myco-estrogen, zearalenone, has attracted recent attention due to the concerns that environmental estrogens have the potential to disrupt sex steroid hormone function. (151). Occasional outbreaks of zearalenone mycotoxins in livestock are known to cause infertility. Alternately, derrivatives of zearalenone are used in some livestock feed for growth promotion (for example, Ralgro in beef cattle), as alternatives to the more potent and controversial synthetic estrogen, diethylstilbestrol. (151). This toxin is found almost entirely in grains and in highly variable amounts ranging from a few nanograms to thousands of nanograms per gram. The appearance of mold on grain plants cannot be relied upon to warn of toxin production because Fusarium infected grain does not necessarily appear visibly moldy in the presence of high concentrations of mycotoxins. (190). The average human intake of zearalenone was estimated to be approximately 0.02 ug/kg bw/d on the basis of limited data obtained in Canada, the United States of America, and Scandinavian countries, but it is likely that intakes are greater in countries from the regions of the world having less well control grain storage systems. (151).

Genotoxicity is a reported concern with respect to zearalenone. Although this estrogenic compound showed no mutagenicity in Ames test (1 to 500 ug/zearalenone/agar plate), the substance induced chromosomal anomalies in some lymphocyte, oocyte, and kidney cell cultures when present within a range of 0.1 to 20 um. (191). This dose range is difficult to extrapolate to likely human exposures because no human bioavailability estimates are available. (151). With estimated human intake of approximately 1 to 2 ug per person, however, occurrence of blood or tissue concentrations remotely close to 0.1M (approximately 30ug/L) seems extremely unlikely. (151).

A related fungal metabolite, alpha zearalenol, which has about 3-fold more estrogenic potency than zearalenone, was recently shown to inhibit atherogenesis, lowering plasma LDL-cholesterol and limiting aortic plaque formation in ovary ectomized rabbits fed on high doses of cholesterol. (151). The effective estrogenic dose of alpha zearalenol was greater than 0.5 mg/kg/d for 12 weeks.(192).

Common human exposure levels for zearalenone related compounds would not be expected to exert such health benefit. (151).

Fumonisms

Fumonisms are produced by the maize pathogens, Fusarium verticilliodes and Fusarium proliferatum, and at very low levels by Alternaria in black and stem rot in tomatoes, asparagus and garlic. (193:194). Maize containing foods are the major concern in the food industry. At least 15 related fumonism compounds have been identified, the fumonism B group is prominent. (151).The Fusarium species comprise a very complicated genus.

The fumonisms are highly water soluble and unlike all other food mycotoxins because they do not have an aromatic structure or a unique chromophore for easy analytical detection. They are primary amines (carbon group substitution at 1 of the 3 bonding positions) with 2 tricarballylic groups, which contribute to their water solubility. Study of the metabolic pathway is in progress. Fumonism synthesis is known to involve an acetate precursor and alanine and several of the genes involved in fumonism synthesis have been identified as a cluster on Chromosome 1 in F. verticilliodes. (195). Fumonism B1 is the agent responsible for leukoencephalomalacia (related to necrotic lesion in the cerebrum) inhorses and pulmonary endema in swine. (151). Hepatotoxicity (liver damage) and nephrotoxicity (kidney damage) have also been reported in connection with fumonism intoxication. (151). Equine leukoencephalomalacia was reported in horses in veterinary medical literature in 1902 but the causative agents were not understood until 1990. Several major outbreaks of these diseases were reported in 1989 and 1990 in the United States of America when the concentration of fumonisms in mid-western maize was quite high. The fumonisms are weakly carcinogenic among different rodent species and are probable human carcinogens associated with increased incidence of esophageal cancers in South Africa and China. (196:197:198:199).

Fumonism consumption is a risk factor in neural tube and related birth defects. (200). Fumonisms are known to disrupt sphinolipid concentrations and synthesis, which may explain the different etiologies of fumonism toxicity in livestock, experimental animal and human. (201). Altered plasma sphingosine/sphinganine (blood serum compounds) ratios serve as biomarkers of fumonism.

(202). Fumonism content in the United States of America corn was relatively high between 1988 and 1991, but has also been low (< 0.5 ug/g) in recent years. There are few reports of high fumonism level (up to 150 ug/g) in home grown corn consumed in China and South Africa. Most commercials, however, contained 500ng/g or less due to low fumonism levels in corn and ingredient quality control. (203).

Grain sorting and sizing have not been shown to reduce fumonism levels in corn. Although corn screenings (small broken pieces of corn kernel) are partly the result of fungal action on the kernels, removal of the screenings did not significantly reduce fumonism levels. (204). Because screenings of Iowa corn elevator samples can account for up to 25% of the corn during 1988 to 1996 this approach for reducing fumonism levels is not economically viable either. (151). Wet milling of contaminated corn (to reduce starch) partitioned fumonisms to all fractions (gluten>fiber>sleep water>germ) except the starch fraction. Fraction yields were not different between fumonism contaminated corn and the control.(205). Dry milling of contaminated corn partitioned fumonism to all fractions, with bran (pericarp) containing the highest level (bran>germ>flour>flaking grits) for white, yellow and blue corn. (206). If levels are reduced sufficiently, the corn may be subjected to decontamination and/or used in low risk product (potentially animal feed). (151). Fermentation of corn to produce ethanol resulted in reduced ethanol yields and did not significantly degrade the toxin. However, distillation of the ethanol resulted in a fumonism free alcohol with fumonisms remaining in the distiller's solid. (207). Ozonolysis of fumonism B resulted in conversion to 3-keto-Fumonism B, however, toxicity was not reduced. (208). Ammoniation has also been investigated without success; treatment of fumonism B containing culture did not reduce its toxicity to rats. (209).

The effects of a variety of heat/chemical processing operations or fumonism content have been investigated and the fumonisms have been proven extremely stable. (151). Researchers investigated the stability of 5ppm in aqueous model systems at pH 4, 7 and 10. They found that fumonism B was most stable at PH 4, however, at temperature above 175 degrees centigrade fumonism B content was reduced by over 90%, regardless, of pH, suggesting that high temperature processing may be effective against

fumonism B in some instances. (210). However, gaps remain in our understanding of thermal effects due to reactions of other food ingredients with primary toxic structural moiety of fumonisms, the primary amine group. The primary amine group is key to detection in chromatographic and immunological analytical methods and can be derivatised with acids/or react with reducing sugars (Maillard reaction) during heat processing, preventing these forms from being detected. The amine group can readily participate in non-enzymatic browning with a variety of reducing sugars and produces an array of typical Maillard products and fumonism B-glucose and fumonism B-fructose adducts, which have been shown to be non-carcinogenic in rat studies. (211:212). Fumonism concentrations are lowered during extrusion processing and the remaining low levels of Maillard reaction products are thought to be detoxified because naturally occurring N-acetyl-fumonism was shown to be non-toxic, and it is presumed that the fumonism glucose adducts are like- wise non-toxic. (213:190). Further investigations of heat application to reduce fumonism B levels included baking and frying, both of which were shown to produce some reduction in fumonism levels. Corn based batter used to make muffins was baked at 175 degrees centigrade and 200 degrees centigrade for 20 minutes, reducing fumonism B levels by approximately 15% to 30%, with increasing losses as temperature increased. (151). Losses were greatest at the surface of the muffins, which was attributed to greater heat penetration as the baking temperature was raised. (214). Frying of masa, to make chips, at temperatures up to 170 degreees centigrade for 6 minutes did not produce significant fumonism losses, frying at 190 degrees centigrade for 15 minutes was required to reduce fumonism B levels by 67%. (213). A more recent study revealed up to 80% reduction in production of fried corn chips, very little of this reduction was the result of frying, however, reduction was due to mixtamillization and rinsing. (215).

Alkaline processing has been shown to be effective in reducing fumonism level and activity. The ester bonds of fumonism B are hydrolyzed to release its tricarballyic groups and yield aminopentol. (216). Overall levels of both fumonism B and hydrolyzed fumonism B were reduced approximately 50% by the traditional mixtamilization process; however both fumonism B and hydrolyzed fumonism B remained in the masa and cooked tortillas in a 1:1 mol/L ratio, suggesting that the cooking, sleeping, and rinsing

processes were effective in reducing toxin content, but that baking to produce tortillas did not result in further reduction. (217). Other studies of this process using a pilot, commercial, and traditional scale process yielded some variation to these results, but most of all exhibited significant reduction of toxicity. Hydrolyzed fumonism B is more bioavailable than fumonism, but less toxic to mice and rats. (218:219:216).Mass production therefore is capable of reducing the toxicity of fumonisms. (219: 216).

Prevention and Control of Mycotoxin in Stored Grains and Seeds

The prevention and control of mycotoxins in stored grains and seeds are associated with three conditions.

1. Drying the grain
2. Avoid grain damage
3. Ensure proper storage conditions

Drying the Grain

Fungi cannot grow (or mycotoxins be produced) in properly dried foods, so efficient drying of commodities and maintenance of the state is an effective control measure against fungal growth and mycotoxin production. To reduce or prevent production of most mycotoxins , drying should take place as soon after harvest and as rapidly feasible. The critical water content for storage corresponds to a water content of about 0.7. Maintenance of foods below 0.7 water content is an effective technique used throughout the world for controlling fungal and mycotoxin production in foods. Problems in maintenance and adequate low water content occur in the tropics where high ambient humidities make control of commodity moisture difficult. Where grain is held in bags, systems that employ careful drying and subsequent storage in moisture proof plastic sheeting may overcome these problems. While it is possible to control fungal growth in stored commodities by control atmospheres or use of preservatives or natural inhibitors, such techniques as drying are thus rarely feasible in developing countries.

Avoid Grain Damage

Damage grain is more prone to fungal invasion and therefore contamination. It is thus important to avoid damage before and during drying, and in storage. Drying maize on the cob, before shelling, is a good practice. Insects are a major cause of damage. Field insect pests and some storage species damage grain on the head and promote fungal growth in the moist environment of the

ripening grain. In storage many insect species attack grain, and the moisture that can accumulate from their activities provides ideal conditions for fungi. To avoid moisture and mold problems it is essential that numbers of insects in stored grain be to a minimum. Such problems are compounded if the grain lacks adequate ventilation, particularly if metal containers are used.

Ensure Proper Storage Conditions

While keeping commodities dry during storage in tropical areas can be difficult, the importance of dry storage cannot be overemphasized. On a small scale, polyethylene bags are effective; on a large scale, safe storage requires well designed structures with floor and walls impermeable to moisture. Maintenance of the water activity of the stored commodity below 0.7 is critical. In tropical areas, outdoor humidities usually fall well below 70% on sunny days. Appropriately timed ventilation, fan forced if necessary, well designed structures with floors and walls impermeable to moisture are adequate. Maintenance of the activity of the stored commodity below 0.7 is still critical.

In tropical areas, outdoor humidities usually fall below 0.7 water content. Ideally, all large scale storage areas should be equipped with instruments for measuring humidity, so that air appropriate for ventilation can be selected. Sealed storage under modified atmospheres for insect control is also very effective for controlling fungal growth, provided the grain is adequately dried before storage, and provided diurnal temperature fluctuations within the storage are minimized. If commodities must be stored before adequate drying this should be for only short periods of no more than, say, three days. Use of sealed storage or modified atmospheres will prolong this safe period, but such procedures are relatively expensive and gas-like conditions are essential. A proven system of storage management is needed, with mycotoxin considerations an integral part of it. A range of decision support system is becoming available covering the varying levels of sophistication and scale involved. It is evident that Silo conditions must be modified in temperate regions because of global warming which can cause external temperatures to increase moisture in the Silo therefore increasing fungal contamination and hence mycotoxin content. It is therefore a need to bypass the Silo.

Detecting Mycotoxins

Mycotoxins occur and exert their toxic effects in extremely small quantities in foodstuffs. Their identification and quantitative assessment thus generally require sophisticated sampling, sample preparation, extraction, and analytical techniques. Two official methods-thin layer chromatography (TLC) and gas chromatography (GC) exist for DON quantification. (220). High performance liquid chromatography methods have been peer reviewed but not yet approved by AOAC International. (221). Despite the availability of approved methods, variability in sampling and testing procedures presents difficulties for precise determinations. (151).

The U.S Federal Grain Inspection Service (FGIS) has evaluated eight commercially available, rapid tests for aflatoxin in maize. FGIS approved kits include rapid ELISA, immunoaffinity cartridge, solid phase ELISA, and selective adsorbent mini-column procedure. There remains a need for efficient, cost effective sampling and analysis methods that can be used in laboratories. One such method could be the use of FTIR Infrared Spectroscopy to detect the presence of mycotoxins in food extracts and the presence of microbial DNA.

Chapter 6

Mushroom Poisoning

Although various wild mushrooms contain an assortment of poisons that are definitely fungal metabolites causing noteworthy health problems in humans, in such cases the distinction is based on the size of the producing fungus and human infection. (161). Mycotoxin exposure is almost always accidental, whereas with mushrooms improper identification and ingestion of the causal mushroom, poisoning is commonly the case. Mushroom poisoning (also known as mycetism) refers to the harmful effects from the ingestion of toxic substances present in a mushroom. The toxins present in mushrooms are secondary metabolites produced in specific biochemical pathways in the fungal cells. Mushroom poisoning is usually the result of ingestion of wild mushrooms after misidentification of a toxic mushroom as an edible species. The most common reason for this misidentification is the close resemblance in terms of color and general morphology of toxic mushroom species with edible species. Even very experience wild mushroom gatherers are upon rare occasion be poisoned by eating toxic species, despite being very aware of the risks, through carelessness. The symptoms of mushroom poisoning can vary from slight gastrointestinal discomfort to death. The period of time between ingestion of the mushroom poison and the onset of symptoms varies dramatically between toxins with some toxins taking days to show symptoms identifiable as mushroom poisoning.

Of the many thousands of mushroom species in the world, only 32 have been associated with fatalities, and an additional 52 have been identified as containing significant toxins. (222). By far the majority of mushroom poisonings are not fatal, but the majority of the fatal poisonings are attributed to Amanita phalloides mushroom. (223:224). Poisonous mushrooms contain a variety of different toxins that can differ markedly in toxicity. Symptoms of mushroom poisoning may vary from gastric upset to life threatening organ failure resulting to death. Serious symptoms do not always occur immediately after eating, often not until the toxin attacks the kidney or liver, sometimes days or weeks later.

Mushroom Toxins and Symptoms

There are eight well studied mushroom toxins, namely:

1. Alpha-amanitin
2. Orellanine
3. Muscarine
4. Gyromitrin
5. Coprine
6. Ibotenic Acid
7. Psilocybin
8. Arabitol

Alpha-amanitin

Alpha-amanitin causes no symptoms for 6 to 12 hours. This is followed by a period of gastrointestinal upset (vomiting and profuse watery diarrhea). This stage is caused by the phallotoxins and typically lasts 24 hours. (222). At the end of this second stage is when severe liver damage begins. The damage may continue for another 2 to 3 days. Kidney damage can also occur. Some patients will require liver transplant.(225). Amatoxins are found in some mushrooms in the genus Amanita, but are also found in some species of Galerina and Lepiota. (226). Overall, mortality is between 10 and 15 percent. (227). Recently, Silybum marianum or blessed milk thistle has been shown to protect the liver from Amanita toxins and promote regrowth of damage cells. (228:229).

Orellanine

Orellanine is a toxin that causes no symptoms for 3 to 20 days after ingestion. Typically around the eleventh day kidney failure begins. (222). All symptoms occur by the twentieth day. These symptoms can include pain in the area of the kidneys, thirst, vomiting, headache, and fatigue. A few species in the very large genus Cortinarius contain orellanine. People having eaten mushrooms containing orellanine may experience early symptoms as well, because these mushrooms also contain other toxins in addition to orellanine. (230). A toxin related to orellanine that causes similar symptoms but within 3 to 6 days has been isolated from Amanita smithiana and some other related toxic Amanitas. (231).

Muscarine

Muscarine stimulates the muscarinic receptors of the nerves and muscles. The symptoms include sweating, salivation, tears, blurred vision, palpitations, and in high concentrations respiratory

failure. (232). Muscarine is found in mushrooms of the genus Omphalotus, also called Jack O' lantern mushrooms. Muscarine and Ibotenic Acid is found in A. muscaria, but the effect of the mushroom is caused by Ibotenic Acid. Muscarine can also be found in some Inocybe species and Clitocybe species, in particular Clitocybe dealbata, and some red pored Boletes. (226).

Gyromitrin

Gyromitrin affects multiple body systems. It blocks the important neurotransmitter GABA, leading to stupor, delirium, muscle cramps, loss of coordination, tremors, and/or seizures. (222). Gyromitrin cause severe gastrointestinal irritation leading to vomiting and diarrhea, and in some cases kidney failure. (222). It can also cause red blood cells to break down, leading to jaundice, kidney failure and signs of anemia. Gyromitrin is found in mushrooms of the genus Gyromitra, and a gyromitrin like compound has also been identified in the genus Verpa. (233: 234). Human stomach acids convert gyromitrin to monomethylhydrazine (MMH), a compound employed in rocket fuel.

Coprine

Coprine is metabolized to a chemical that resembles disulfiram. Coprine inhibits aldehyde dehydrogenase (ALDH), which in general, causes no harm, unless the individual has alcohol in his/her bloodstream while ALDH is inhibited. This can occur if alcohol is ingested shortly before or up to a few days after eating the mushrooms. In this situation alcohol cannot be completely metabolized, and the person will experience flush skin, vomiting, headache, dizziness, weakness, apprehension, confusion, palpitations, and sometimes trouble breathing. Coprine is found mainly in mushrooms of the genus Coprinus, although similar effects have been noted after ingestion of Clitocybe clavipes.

Ibotenic Acid

Ibotenic acid decarboxylates into muscimol upon ingestion. The effects of muscimol varies, but nausea and vomiting are common. Confusion, euphoria, or sleepiness are possible. Loss of muscular coordination, sweating and chills are also possible. Some individuals experience visual distortions, a feeling of strength, or dilutions. The symptoms normally appear after 30 minutes to 2 hours and for several days. A. muscaria, the "Alice in Wonderland' mushroom is known for the hallucinatory experiences caused by muscimol, but A. pantherina and A. gemmata also contain the

same compound. (226). The consumption of a large number of any of these mushrooms is likely to be dangerous, though normally self- limiting. Fatalities have been associated with A. pantherina. (235).

Psilocybin

Psilocybin dephosphorylates into the psychoactive psilocin upon ingestion, which acts as a psychedelic drug. The symptoms begin shortly ingestion. The effects can include euphoria, visual and religious hallucinations, and heightened perception. However, some individuals experience fear, agitation, confusion, and schizophrenia-like symptoms. All symptoms generally pass after several hours. Some members of the genus Psilocybe contain psilocybin, as do some members of other genus such as Panaeolus, Copelandia, Conocybe, and Gymnopilus. Baeocystin is also found in some of these mushrooms and have effects similar to psilocin.

Arabitol

Arabitol is a large sugar alcohol, similar to mannitol, which causes no harm to individuals but cause gastrointestinal irritation in some. Arabitol is found in small amounts in oyster mushrooms, and in considerable amounts in Suillus species and Hydrophoropsis aurantiaca (the false chanterelle mushroom). (236). Some mushrooms contain less toxic compounds and therefore are not severely poisonous. Poisonings by such mushrooms may respond well to treatment. However, certain types of mushrooms, such as the Amanitas, contain very potent toxins and are very poisonous, so even if symptoms are treated promptly mortality is high. With some toxins death ca occur within a week or a few days. Because some mushrooms contain more than one toxin the symptoms can be multifactorial corresponding to the various toxins.

Although a liver or kidney transplant may save some patients with complete organ failure, in many cases there no organs available. Patients hospitalized and given aggressive support therapy almost immediately after ingestion of amanitin containing mushrooms have a mortality rate of only 10%, whereas those admitted 60 or more hours after ingestion have a mortality rate of 50 to 90%. (233).

Chapter 7

Parasites in Foodborne Disease

The major parasites of importance in food borne diseases are Cryptosporidium, Giardia spp., Cyclosporia, Toxoplasma, Scarcocystis, Microsporidia, Trematodes, Cestodes, Nematodes, and Entamoeba histolyyica. Some of these parasites in addition to causing food borne disease are also water borne. This water borne aspect means that they can cause disease either by direct consumption of the infected water by humans or by crop irrigation with the contaminated water.

Cryptosporidium

Cryptosporidiosis is a parasitic disease caused by Cryptosporidium, a parasite of the phylum Apicomplexa. The disease is also known as "Crypto'. (237). Of the eight species of Cryptosporidium, all of which infect vertebrates, only one, Cryptosporidium parvum, is zoonotic. (238). Cryptosporidium causes diarrhea in livestock and humans, and the organism was first recognized in asymptomatic laboratory mice. (239:238). The economic importance of the parasite became apparent to the agricultural world when an outbreak of severe diarrhea was reported in 1955 in turkeys and a similar problem was reported in calves in 1971. (238). Since then, the parasite has been associated with outbreaks of diarrhea in young cattle, sheep, goats, deer, horses, dogs, cats, and turkeys. (238). Cryptosporidium parvum is the important protozoa that cause diarrhea in humans and animals. (240). Some Cryptosporidium parvum strains show a zoonotic transmission whereas others show an anthroponitic transmission. (240). Infected hosts can excrete oocysts, and both domestic and sylvatic animals may be reservoirs for human infection. (240).

The environment can be contaminated through direct deposit of animal and human feces or through the discharge of sewage and waste water into waters. (240). Hence, the consumption of contaminated food (agricultural products, such as fruits and vegetables, undercooked or cross contaminated meat) and water can cause disease. Human infections may result from the ingestion of 30 Cryptosporidium oocysts. (241). Under favorable conditions of temperature and humidity, oocysts can remain viable for several

months increasing the probability of the occurrence of infection. (242:241).

Diarrhea is the most common symptom of C. parvum, followed by abdominal pain and vomiting. (239). Cryotosporidium is transmitted through the fecal oral route. (243:244). Symptoms appear from two to ten days after infection, with an average of seven days, and can last up to two weeks, or in some cases up to one month. (237). There are three possible forms of illness in immunocompetent people; the disease can be tragic or cause acute diarrhea or persistent diarrhea that can last for a few weeks; diarrhea is watery with mucus and it is very rare to find blood or leukocytes in the diarrhea. (245). Pancreatitis can occur in immunocompetent individuals. (245).

Immunocompromised individuals, s well as the very young or very old can develop a more severe cryptosporidiosis. (247). There are four clinical presentations for patients with aids; 45 have no symptoms, 295 have transient infection, 60% have chronic diarrhea, and 8% have severe cholera like infection. With transient infections diarrhea ends within 2 months and Cryptosporidium is no longer found in the feces. Chronic diarrhea is diarrhea that lasts 2 or more months, and results in patients excreting at least two liters of watery diarrhea per day. (245). Aids patients can have up to 10 stools per day, experience mal-absorption, and have 105 weight loss. Many of them can never completely eliminate Cryptosporidium from their bodies. (245). When Cryptosporidium spreads beyond the intestine, as it can in aids patients, it can reach the lungs, middle ear, pancreas, and stomach, with one symptom being pain in the right quadrant. (248). The parasite can infect the bilary tract, causing bilary cryptosporidiosis, that can result in cholecystitis and cholangitis. (249).

Some of the diagnostic tests for Cryptosporidium include Microscopy, Staining and the detection of antibodies. (237). Microscopy can help identify oocysts in fecal matter and at least 3 stool samples should be tested. (247:250). Fluorescent Microscopy is done by staining with auramine. (247). Methods used to concentrate the stool sample or oocysts are the modified formalin-ethylacetate method, the modified zinc sulfate centrifugal flotation technique and Sheather's sugar flotation procedure. (251:250). Other staining techniques include acid fast staining, which stain the oocysts red, Giesma staining and the Kinyoun technique.

(248:251:249). The detection of antigens to diagnose the disease can be achieved with the direct fluorescent antibody (FDA) technique, indirect immunofluorescent assay, and enzyme-linked immunofluorescent assay. (237:250:247). Polymerase chain reaction (PCR) is another procedure for the diagnosis of Cryptosporidiosis. It can identify specific species of Cryptosporidium. (252). If the patient is thought to have bilary cryptosporidiosis, then the appropriate diagnostic technique is ultrasonography. If that returns normal results, then the next step would be to perform endoscopic retrograde cholangiopancreatography. (248).

Cryptosporidium is highly resistant to chlorine disinfectant, but with high enough concentration and contact time, Cryptosporidium will be inactivated with chlorine dioxide and ozone treatment; soaked contaminated surfaces can be decontaminated with 3% hydrogen peroxide after 20 minutes of treatment. (253:254). Ultraviolet light treatment in low doses will inactivate cryptosporidium. (255). The most reliable way to decontaminate drinking water that may be contaminated with Cryptosporidium is to boil it (256:257). The best way to prevent getting and spreading Cryptosporidiosis is to have good hygiene and sanitation. (250). Examples would be hand-washing; avoiding possible contaminated food and water; and individuals should refrain from engaging in several activities that can expose them to feces. (250). Water can also be made safe by filtering with a filter with pore size not greater than one nanometer. (237). Bottle drinking water is less likely to contain Cryptosporidium if it is from an underground source. (258). Heating milk at 71.7 degrees centigrade (161F for 15 seconds pasteurizes it and can destroy the oocysts' ability to infect. (258).

Individuals with cryptosporidiosis should not swim in communal areas because the pathogen can reside in the anal and genital areas and be washed off in the water. They should also stay away from immunocompromised individuals. Immunocompromised individuals should protect themselves from water in lakes and streams. (248). They should stay away from animals and wash their hands after touching animals. To be safe, all individuals should use boiled or filtered water, and wash and/or cook their agricultural produce such as fruits and vegetables. (237).

Cryptosporidiosis has been associated with unpasteurized apple juice and unwashed salad onions. (239).

There is no reliable treatment or vaccine available for Cryptosporidium. (238). However, certain drugs such paromomycin, atovaquone, nitazoxamide, and azithromycin are sometimes used, but they usually have temporary effects. (259). Cryptosporidiosis is a disease that is found in both developing and developed countries. (238).

Giardia

Giardia infects humans and other animals and the organism causing giardiasis is Giardia duodenalis, sometimes referred to as Giardia lamblia or Giardia intestinalis (regarded by some as a race of Giardia duodenalis. (239). For the purpose of this article the organism will be referred to as Giardia lamblia. Other species for example, Giardia muris only infect other animals (rodents, birds and reptiles). (239). Giardia infection is the most common intestinal parasitic disease in the United States of America. About 2% of adults and 6% to 8% of the children in the United States of America and other developed countries have had the Giardia infection. In contrast up to one third of people in developing countries have been infected. (237). Various genetic variants of Giardia infect humans and animals world-wide. (238).

The infection caused by Giardia is known as Giardiasis. The initial signs of the illness associated with giardiasis include nausea, anorexia, discomfort in the upper intestine, and fatigue. (238). This is followed by bursts of fowl-smelling watery diarrhea, lasting only a few days. (238). This acute stage can sometimes last for months causing malabsorption, weight loss and debilitation. (259:260). Chronic infection is marked by recurrent brief episodes, or to a lesser extent, persistent episodes of fowl-smelling loose stools, flatulence, and abdominal distention. (238). Lactose intolerance is common during active infection and may persist for a period thereafter. Mortality has rarely been reported for the disease. (238).

The time between infection and the appearance of Giardia cysts in the stool is 12-19 days. (261). Symptoms appear between 7 and 75 days after infection, but generally at 6-15 days, coinciding with the appearance of Giardia in stool. (262:263). The most prominent symptoms are diarrhea (fatty yellowish), weakness, weight loss, abdominal pain, and to a lesser extent nausea, vomiting, flatulence

and fever. (264). However, a significant proportion of the infected population (estimated at 30-50%) will then develop chronic infection with intermittent diarrhea. (265). Weight loss can be substantial (10-20%) in this group. (264). The ability of Giardia to change the surface epitopes of the trophozoites during infection may play a role in chronic infection. (264:266). Evidence exists that the infection of children with Giardia causes failure to thrive by impairing the uptake of nutrients, especially fats, and Vitamin A and B12. (265:267). Excretion of cysts varies between 1,000,000 and 10,000,000 per gram of stool, as was determined in positive stool samples, but a significant proportion of the stool samples do not contain detectable levels of Giardia. (268:264). Excretion pattern varies with the host and with the isolate. (264).

Diarrhea caused by Giardia must be differentiated from other entero-pathogenic agents by diagnosis. (238). Some clinical indications for giardiasis are provided by the presence of upper abdominal cramps, abdominal distention, flatulence, steatorrhea and fowl-smelling feces and by the absence of blood and mucus in the feces. (238). Traditionally, diagnosis is based on the identification of cysts or less frequently, trophozoites in feces, or trophozoites in duodenal aspirates or biopsis. (238). Most diagnosis can be performed by stool examination. Fresh stools must be examined immediately by wet smear or preserved in buffered formalin solutions or methiolate iodine formalin. (238).At least three stools collected on alternate days should be examined before other procedures for diagnosis are attempted. When organisms in fecal smears are absent, zinc sulfate solution should be used for floatation and concentration; sugar and other salts should not be used because these distort the parasite, rendering it unrecognizable. (260). When Giardia can not be detected in stools of suspected cases, duodenal or jejunal fluid should be obtained and exmined by duodenal tube, endoscope or duodenal contnt sampling by the duodenal string capsule technique (entero-test). (238). These traditional diagnostic methods either lack methodical sensitivity, are difficult to perform, or involve invasive procedures, and hence comprehensive immunological methods have been developed to support diagnosis. (238). Serodiagnosis by the detection of anti-Giardia antibodies is not appropriate. (238). Conversely, immunodiagnostic detection of Giardia antigen, including immunodiffusion, immunofluorescence,

countrimmunoelectrophoresis and ELISA, have shown great value and have resulted in a large commercial market for rapid and reliable tests. (269). In endemic non-industrialized countries where stool microscopy may provide the only diagnostic option, therapeutic trial may be justified when clinical symptoms suggest Giardia infection but repeated stool samples remain negative.

The treatment of choice for humans varies according to the country, due to the differences in approved drugs. (238). Generally, giardiasis is treated with nitroimidazoles, such as metronidazole, ornidazole or tinidazole or albendazole. (238). Furazolidone has been recommended for the treatment of children. Paromomycin has been prepared for the treatment of pregnant women. (238). In domestic animals, especially in dogs, the drugs used are the same as for humans, except that fenbendazole has been demonstrated to be highly effective in dogs, (other animals not included). (238). Indication for treatment is usually restricted to symptomatic companion animals. Symptomatic infections in livestock are rare and are usually self-limiting and self-healing. (238).

Fecal-oral transfer of Giardia cysts is the major route of transmission of giardiasis, as indicated by the high prevalence in developing countries with poor standards of hygiene and sanitation, in day care centers and nurseries, and spread within the house hold of those who attend day care centers. (270:271:272). Food borne outbreaks are the result of contamination of food by infected workers or house hold members. (273:274:275). The consumption of contaminated agricultural produce such as fruits and vegetables is possible is contaminated irrigation water is used. Giardiasis has been associated with salmon, noodles, fruit, salad and sandwiches. (239). Water borne outbreaks have been reported for some 30 years world-wide. (276:263:277). These outbreaks have been linked to consumption of untreated surface water contaminated by human sewage, or by wild rodents, or by ground water that was contaminated by sewage or contaminated surface water. (277:276:278).

The role of animals in the transmission of human giardiasis is still a matter of some speculation. (264). Although Giardia commonly occur in domestic pets, farm animals, and wild mammals, there is no unequivocal evidence that organisms from these sources have caused infections in humans. (279). Giardia lamblia isolates from animals and humans may be morphologically indistinguishable, and

this has led to numerous reports of animal sources of human giardiasis, including water borne infections caused by Giardia cysts from beavers and muskrats. (280:276:278). However, the genetic diversity within and between human and animal isolates is too high to allow definite conclusions to be drawn regarding host specificity. (281:264). Cross transmission studies have not been well controlled and results have been contradictory. (282:283:284:285:286:264).

The most important measures to prevent giardiasis are personal hygiene, proper sanitary infrastructure, and the appropriate treatment of drinking water, such as proper filtration and boiling. Given the potential for virtually all surface water to be exposed to Giardia contamination from human and animal sources, caution dictates that all cysts of Giardia be regarded as potentially infective for humans. (238). Special guidelines, recommendations, and regulations for controlling water borne Giardia have been developed or proposed by national and international organizations. (260).

Cyclospora

Many species of Cyclospora have been identified in animals. (239). Cyclospora cayetanensis is the only species found in humans, and it is apparently restricted to this host. (239:264). The parasite was initially recognized as a human pathogen in 1977. (287). The oocysts of Cyclospora are shed in feces. (239).

The incubation time for Cyclospora, from the ingestion of oocysts to the onset of symptoms is between 2 and 11 days, but typically about 1 week. (239: 288). The symptoms of Cyclospora infection include watery diarrhea, fatigue, and loss of appetite, weight loss, vomiting, low grade fever, and stomach cramps. (239:264). The illness may last for weeks and episodes of watery diarrhea may alternate with constipation. (288). In immunocompetent individuals the symptoms are self-limiting and oocysts excretion is associated with the clinical illness. (289). The disease typically lasts for 2 weeks in immunocompetent individuals. (239). In immunocompromised individuals diarrhea may be prolonged. (264).

Epidemiological data indicate that Cyclospora spp. Are transmitted by water and food. (290:291:292). It is likely that the most significant transmission may occur where sewage or water contaminated by human sewage effluent can effect humans or contaminated crops. (239). Cyclospora infection has occurred as a

water borne as well as a food borne disease. (293). Cyclosporiasis has been associated with raspberries, mixed baby lettuce, basil, and salads. (239). A significant reservoir of Cyclospora cayetanensis has yet to be identified in animals. (239). Oocysts of Cyclospora spp. Have been isolated from the stools pf children and both immunocompetent and immunocompromised individuals. (264). The disease occurs in both developed and developing countries. The clinical disease may be resolved without treatment, but the treatment drug of choice is trimethoprim-sulfamethoxazole. (264:239).

Toxoplasma

The disease "Toxoplasmosis" is caused by the microscopic parasite Toxoplasma gondii. Toxoplasma gondii is possibly the most widespread and prevalent protozoan parasite on Earth, infecting approximately half a billion people. (260). The range of prevalence reported world-wide is great and may locally depend upon meat consumption habits and the population density of cats. (238).

The initial clinical signs of toxoplasmosis are usually characterized by non-specific and variable symptoms resembling those of influenza. (238). Acute infections include dermatomyositis, encephalitis, enteritis, hepatitis, lymphadenitis, myocarditis, placentitis, pneumonitis, retinochoroiditis, skeletal myositis, tenosynovitis, tonsillitis, vasculitis, anemia and fever. (260). The period of incubation is variable and in most cases, clinical signs are manifested a few days to a few weeks after exposure. (238). The duration and severity of the illness is very limited among immunocompetent individuals. In individuals with cancer or Acquired Immune Deficiency Syndrome (AIDS), infections of long duration, followed by death is possible. (238). An at risk group of special interest is non-immune pregnant women. In Europe, congenital toxoplasmosis is reported 0.15 to0.2% of pregnancies where the mother is seronegative prior to or during early pregnancy. (294).

In humans and animals, direct diagnosis is based on medical history, tissue sections, tissue biopsies, fluid aspirates, or other diagnostic substrates that may contain Toxoplasma gondii organisms. (238). The detection of parasites can be performed using microscopical, histological, or immuno-histochemical techniques. (238). Because parasites can be few in number, these approaches have low sensitivity. (238). Alternatively, the diagnostic

amplification of Toxoplasma gondii deoxyribonucleic acid by the Polymerase Chain Reaction (PCR) has been used successfully in investigations of the parasites in both humans and animals. (295:296). The major serological tests include direct agglutination tests, the modified agglutination tests, the Sabin Feldman dye test, the indirect antibody test, and the Enzyme Linked Immunosorbent Assay (ELISA). (238:297). Agglution tests and ELISA are available, but the latter is offered to detect immunoglobulins IgM, IgG, and IgA, and even to assess the acidity of the antibody binding reactivity in order to discriminate between recent (acute) and old infections. (238:297). Serology is reliable as a diagnostic tool for animals if blood samples are used with the modified agglutination test. (297). There is no serological test for meat to date. (238).

Toxoplasma gondii infection is generally transmitted either congenitally or via ingestion of undercooked or raw meat from infected animals, or the ingestion of food or water contaminated with oocysts excreted by infected felids (cats). (298:299:300). Although a major source of infection is thought to result from contamination of the environment with oocysts shed in cat feces, the extent of human infection resulting from this route is unknown. (238). Feces are deposited in gardens, fields, lawns, pastures, playgrounds and other locations available to cats. (238). The oocysts are microscopic in diameter and are dispersed easily by wind, water, shoes, and feet of human and animals and numerous other routes. (238). Transmission of Toxoplasma gondii by ingestion of tissue cysts in raw or undercooked meat from a variety of livestock and game animals has been documented as another major source of human infection. (301). Toxoplasma gondii has been found in market sold adult chickens, ducks, and pigeons in China. (297). Specific cultural preferences for raw or undercooked meat inevitably result in a higher prevalence of infection. (238).

Pyrimethamine with sulphonomides acts synergistically against the rapidly dividing tachyzoite stage and is the treatment of choice in many countries. (238). Spiramycin, clindamycin and macrolide antibodies are also reported to be effective treatments. (238).All the drugs used above have serious side effects and should be used with caution. Measures that reduce or eliminate contamination of the environment with oocysts from cats will help prevent toxoplasmosis. (260). Cats should be prevented from hunting birds and rodents, and should be fed thoroughly cooked,

dry or canned food. (238). All meat should be thoroughly cooked before eating. (238).

Sarcocystis spp.

Sarcocystis spp., are coccidian protozoans that cause sarcocystiosis and have a global distribution. (238). Numerous species of Sarcocystis infect cattle, sheep, pigs, horses, camels, buffalo and wild game animals. (238). Heavy infections in the above mentioned intermediate hosts may cause abortion, anorexia, fever, anemia, and reduce live weight gain. Two species are recognized to be zoonotic, namely, S. hominis and S. suihominis, and are found in humans. (302). Sarcocystis honinis is mildly pathogenic in humans causing stomach pains, nausea and diarrhea. (238). S. hominis is acquired in humans by the consumption of undercooked beef containing zoitocysts. Sporocysts begin to be excreted in the feces after 14-18 days with S. hominis and 11-13 days after infection with S. suihominis. (238). S. suihominis is acquired by eating zoitocysts in undercooked pork. (238). S.suihominis is more pathogenic than S. hominis, and causes symptoms such as stomach pains, nausea, diarrhea, and dyspnea within 24 hours of infection. (238). There is no effective treatment for the infection, and the best control method is to avoid eating undercooked meat. (238).

Microsporidia

Microsporidia are obligate intracellular parasites that are opportunistic pathogens among both immunocompromised and immunocompetent individuals. (303). Human microsporidiosis is a serious disease. Which currently occurs more frequently than in the past in immunocompetent and immunocompromised individuals with the most common species identified as Encephalitozoon intestinalis, Encephalitozoon hellum, Encephalitozoon cuniculi, and Enterocytozoon bieneusi. (303).The symptoms of microsporidiosis include frequent intestinal infections and extraintestinal infections such as urinary tract, respiratory tract, disseminated systematic infections, sinusitis, otitis and keratoconjunctivitis. (304:303).

Despite the advances in molecular technology, most epidemiological aspects of human microsporidiosis, particularly transmission cycles remain unresolved. (303). Direct-contact exposure, food borne and water borne transmissions are postulated to be predominant. (305:306:307:303:308:309). Microsporidia are on the contaminant candidate list of the United

States Environmental Protection Agency because their transmission routes are unknown, spore identification, removal, or inactivation in drinking water is technologically challenging, and infections are difficult to treat. (310:303:311). The identification of microsporidian spores known to infect humans represents a challenge because microsporidia can infect a variety of non-human hosts, and spore morphology is insufficient for species identification. (303).

Chapter 8

Trematodes

Trematodes are a class of parasitic flat worms comprising the flukes, such as Fasciola hepatica, Clonorchis sinensis, Opisthorchis viverrini, Opisthorchis felineus, Heterophes heterophyes, Metagonimus yokogawai, Paragonimus westermani, Paragonimus miyakii, Paragonimus skryjabini, Paragonimus heterotrema, Paragonimus africanus, Paragonimus interobilateralis, Paragonimus mexicanus, Echinostroma ilocanum, Echinstroma revolutum, Echinostroma malayanum, Echinostroma echinatum and Echinostroma hortense.

Fasciola hepatica

Fasciola hepatica, the common liver fluke, affects predominantly ruminants and occurs throughout most of the temperate regions of the world. (238). Humans are rarely affected, although the World Health Organization (WHO) estimated that 2.4 million individuals are affected world-wide. (312). Other fascolid flukes may also infect humans but are considered to be of minor importance. (238). When the disease occurs it is related to the intensity of exposure and the stage and duration of the infection. (238). Most cases remain sub-clinical, but when symptoms occur, they are related to the hepatobiliary system. (238). Sometimes, the aberrant migration of immature flukes leads to unusual ectopic manifestations. (238).

In established infection, Fasciola hepatica can be diagnosed by detecting parasite eggs in the feces. If the eggs are absent, such as in the early tissue invasive stage, or in exclusively ectopic disease, serology may be useful through antigenic components. (313). Blood eosinophilia supports the diagnosis, but not always present. (238). Non-invasive imaging techniques may help to establish the extent of liver damage, but do not specifically confirm the diagnosis. (314). The biopsy of ectopic lesion provides a definitive diagnosis. (238). Very occasionally, eggs appear in feces as a result of gastro-intestinal passage in individuals who consume infected sheep liver. (238).

Most individuals become infected by eating wild watercress or other fresh water raised plants. However, infective metacercarie may, in some cases, be acquired directly through drinking water. (238). The drug of choice for the treatment of Fasciola hepatica

infection is triclabendazole, which is given in a single dose of 10mg/kg body weight. (315). Another drug Praziquantel has not proved effective in treating Fasciola hepatica infection. (238). In ectopic disease surgery is both diagnostic and curative, and chemotherapy is not useful. (238). The disease can be prevented by avoiding the consumption of raw leaf water grown vegetables and contaminated water, especially if the water is obtained from ponds or streams exposed to sheep and cattle feces. (238).

Clonorchis sinensis, Opisthorichis virverrini, and Opisthorchis felineus

There are human flukes which are also parasites of fish eating mammals. (238). There are predominant in Asia and Europe where more than 20 million individuals are infected. In highly endemic areas such as North-east Thailand the prevalence of Opisthorchis viverrini may reach 90%. (316). Clonorchis sinensis is highly prevalent in the People's Republic of China, Taipei China, Vietnam, and still occurs in Japan and the Korean peninsula. (238). The main animal hosts including humans are cats, dogs, pigs, rats, and camels. (238). Opisthorchis felineus is prevalent in Poland, Eastern Germany, and parts of the former Soviet Socialists Republics. (238). The adult flukes live in the bile ducts and can occur in large numbers. Large flask shape operculated eggs are shed in feces and hatch if ingested by the appropriate intermediate snail host. (238). Cercariae are shed which penetrate the skin of fish of the family Cyprinidae, and eventually encyst in the muscles of these fish. (238).

Infectionoccurs through eating undercooked or raw fish and the metacercariae reach the liver by direct migration up the bile duct. (238). Raw fish dishes are a dietary habit in all countries where these flukes are normally found. Clinical manifestations are rare, but infections increase the risk of cholangiocarcinoma. (317). Control is possible through the treatment of the infections with praziquantel, sanitation measures, and population education to discourage the consumption of raw fish. (238).

Heterophyes heterophyes and Metagonimus yokogawai

These are probably the two most important of the numerous different species of small, zoonotic intestinal flukes found in mammals and birds which ingest encysted metacercariae in raw or undercooked fish. (238). The fluke of these two species commonly occur in Japan, Laos, Thailand, the Republic of Korea, Hawaii, the

Balkans, the Philippines, The People's Republic of China, Taipei China, Turkey and Siberia. (238). The operculated eggs are passed in feces and are morphologically very similar. Clinical manifestations are rare and, if present, are usually due to eggs trapped in the various tissues. (318). The drug of choice for treatment is praziquantel, and control include sanitation measures, and education to discourage the consumption of raw fish. (238).

Paragonimus westermani, Paragonimus miyazakii, Paragonimus skryjabini, Paragonimus heterotrema, Paragonimus africanus, ***Paragonimus interobilateralis and Paragonimus mexicanus***

These are lung dwelling flukes that cause the infection known as paragonimosis, and infect over 20 million individuals world-wide. (238). Countries with a significant number of cases include the People's Republic of China, Taipei china, Thailand, Japan, Nigeria, Cameroon, Peru and Ecuador. (238). In addition to humans the definitive hosts include dogs, cats, and wild carnivores. Aquatic snails are the first intermediate hosts from which cercariae are released, subsequently infecting crabs, crayfish or shrimp. (238). Human are infected by eating raw or undercooked crabs, crayfish, or shrimp which are infected by encysted metacercariae. Infection may also occur when paratenic hosts such as wild boar are consumed. (238).

The symptoms of the infection include a chronic productive cough and chest pain that is characterized as a pulmonary infection. Other clinical signs, when combined with an appropriate history, are relatively pathonomonic. (238). In addition to the lungs, these flukes can be found in the brain, giving rise to neurological symptoms. (238).

Diagnostic measures include the detection of eggs in sputum or feces and the use of dot-ELISA tests. Plain radiographs are useful for detecting pulmonary masses. The majority of neurological infections are detected by computerized tomography (CT) scans which show brain lesions. The drug of choice for treatment is praziquantel and control measures include education to avoid eating raw or undercooked crabs, crayfish, shrimp or wild boar.

Echinostroma ilocanum, Echinostroma revolutum, Echistroma malayanum, Echistroma echinatum and Echistroma hortense

These flukes are of minor zoonotic importance but a high prevalence has been reported in endemic countries such as the Republic of Korea, the Philippines, Indonesia, Malaysia, and

Thailand. (319). Humans can become infected by ingesting metacercariae encysted in fresh water snails and fish. (238). Symptoms of heavy infection include diarrhea, anorexia and abdominal pain. The eggs are shed in feces. The drug of choice for treatment is praziquantel and control measures include education to avoid eating undercooked or raw fresh water fish.

Chapter 9

Cestodes

Cestodes are also called tapeworms. Adult cestodes are typically long, flat, segmented worms that lack a digestive tract and absorb nutrients directly from the host's small intestine. (320). An adult tapeworm has three recognized portions. The scolex or head which functions as a holdfast organ. The neck, which is an unsegmented region of high regenerative capacity, and the rest of the worm that consists of numerous segments (proglottides). The segments closest to the neck are undifferentiated, and as the proglottides move caudally the hermaphroditic sex organs are formed. The distal segments are gravid and contain eggs in the uterus. The main species of economic and medical importance to humans are Taenia solium (pork tapeworm), Taenia saginata (beef tapeworm), Diphyllobothrium latum (fish tapeworm), and Echinococcus spp.

Taenia solium (Pork Tapeworm)

Taenia solium has cosmopolitan distribution being more wide spread in Latin America, Africa, and Asia (India). Taenia solium cysticercosis is reported world- wide. (238). The health importance of T. solium cysticercosis has been seriously underestimated in numerous regions of the world. (321). World-wide the number of deaths due to neurocysticercosis is estimated at approximately 50,000 (322).

Individuals infected with, and carrying T. solium experience symptoms such as abdominal pain, nausea, weakness, loss or increase appetite, headache, constipation, diarrhea, anal pruritus, hyperexcitability and neurocysticercosis with seizures and epilepsy. (238:320). Symptoms due to neurocysticercosis in humans vary considerably according to the number and location of cysticerci, the status of the cysticercus (living or dead), and the immune response of the host. (323). In many individuals the infection may remain asymptomatic despite long-term and even severe infections. (238). T. solium proglottides are generally eliminated with the feces, hence T. solium infections may go unnoticed. (238).

The classical coprological techniques (CT) with repeatations, the perianal swab technique, the ELISA test and DNA probes or PCR assays are used to diagnose T. solium infection. (238). Contrasted CT and Magnetic Resonance Imaging (MRI) are the techniques of

choice to confirm neurocysticercosis. (322). Using purified glycoproteins of T. solium a highly sensitive and specific immunoblotting technique has been developed to aid in diagnosis. (324).

The drugs of choice for the treatment of T. solium infections are praziquantel or niclosamide. Albendazole and to a lesser extent, praziquantel, are considered the treatment of choice for cysticercosis in humans especially when steroid drugs are used simultaneously. (323). The eradication of T. solium infection and cysticercosis is possible in humans for the following reasons.

1. T. solium infections in humans are the only source for intermediate hosts.
2. Domestic animal intermediate host populations can be managed to avoid infection.
3. There is no significant wild life reservoir
4. Thoroughly cooking pork eliminates the chance of infection
5. Effective treatment is available for T. solium infection. (238;322).

Taenia saginata (Beef Tapeworm)

Taenia saginata has cosmopolitan distributions with the organism being prevalent in the cattle raising regions of the tropics and sub-tropics in Africa, the Middle East, Eastern Europe, Mexico and South America. The organism is not very common in the United States of America and is monitored by Federal Inspection. T.saginata is less researched than T. solium. However, the most important source of T. saginata eggs for lightly infected farms appeared to be the use of effluent from sewage treatment plants as drinking water for cattle, whereas the origin of infection in heavily infected farms was related to the use of sludge from septic tanks. (325).

The symptoms of T.saginata infection are abdominal pain, nausea, weakness, loss of appetite or increase appetite, headache, constipation, dizziness, diarrhea, anal pruritus, and hyperexcitability. (238:320). The T. saginata proglottides are normally passed in or outside the stools. (238). The diagnosis of T. saginata infection include classical coprological techniques with several repetitions for the detection of the tapeworm in faces, the perianal swab technique, the ELISA test for the detection of parasite antigens in stool, and DNA probes or PCR assays. (238). The drugs of choice for the treatment of T. saginata infection are

praziquantel or niclosamide. The control measures are to educate individuals about the danger of eating undercooked beef and monitor and treat cattle for infection.

Diphyllobothrium latum (Fish Tapeworm)

Diphyllobothrium latum is a group of tapeworms that inhibit the human intestinal tract. The organism is found world-wide with the major foci in the fresh water lakes of Europe, in areas of the former Union of Soviet Socialists Republics, Finland, Scandinavia, the Alpine zone, and in Asia and the United States of America. The D. latum infection is usually asymptomatic. In some cases mild gastrointestinal obstruction, rarely diarrhea and abdominal pain, ocassional leukocytosis with eosinophilia, and vitamin B12 deficiency which cause anemia is present.(238:320). Egg production starts approximately 30 days after the infection and vitamin B12 deficiency is caused by the high affinity of the tapeworm for the vitamin.

As the infection is acquired by the consumption of raw fresh water fish, preventative measures include thoroughly cooking of fish and treatment with praziquantel. (238). Sometimes vitamin B12 is administered to correct the anemia. (320). In areas with high fresh water fish and carnivore population densities, regular treatment with praziquantel of domestic carnivores, especially those being fed raw fish, can reduce environmental contamination of natural water resources with parasite eggs. (238).this may be particularly useful in relation to fresh water aquacultures, and preventing wild carnivores access to facilities by appropriate fencing may also provide good results. (238).

Echinococcus spp.

Echinococcosis is a group of infectious diseases caused by the tapeworms of the genus Echinococcus. The two most important species associated with public health are, Echinococcus granulosus, the organism that causes cystic echinococcosis (CE), and Echinococcus multilocularis, the causative organism of alveolar echinococcosis (AE).

Echinococcus granulosus

Infections with E. granulosus occur throughout the world. E. granulosus is a small tapeworm found in the small intestine of carnivores, predominantly dogs. (238). Ungulates and rarely some other animals and humans are intermediate hosts. In the definitive hosts sexual maturity of the adult tapeworm is reached within five

to six weeks. (238). Gravid proglottides, each containing several hundred eggs, and eggs liberated from disrupted proglottides are shed in feeces. (238). Following the ingestion of E. granulosus eggs by susceptible intermediate hosts, a primary larva (the oncosphere) hatches from the egg and penetrates the intestinal epithelium into the lamina propira, and the oncosphere is then transported to primary target organs such as the liver, lungs and other organs. At these sites hydatid cysts maturation occur which may result in the formation of protoscoleces. (238).

Cases of cystic echinococcus (CE) are usually present with well delineated spherical primary cysts. (326). Although variations occur due to geographic locations, approximately 65% of cysts occur in the liver, 25% in the lungs, and the other 10% can occur in almost any other location. (326). Cysts cause pathological damage in, or dysfunction of, infected organs mainly by the gradual process of space occupying repression or displacement of vital organs, tissues or vessels. (238). Cyst rupture may be a cause of anaphylactic or acute inflammatory pathology. (238). Clinical manifestations are primarily determined by the site, size, and number of cysts. (238). Success with surgical removal of hydatid cysts is variable, depending on the facilities. In countries with modern medical facilities, the fatality rates are low, varying between 1% and 4% for first surgical intervention cases. (326). The mortality rate is much higher in countries where operating and post- operative care facilities are not optimal. (238).

For the diagnosis of E. granulosus larval infections in humans imaging procedures together with serology are the diagnostic tools used. (238). Ultrasonography (US) is the primary diagnostic procedure of choice. (238). For the detection of extrahepatic disease and volumetric follow-up assessment computerized tomography (CT) is the superior technique whilst MRI adds diagnostic benefit by identifying changes of the intra- and extra-hepatic venous system. (238). Ultrasonography is also useful in longitudinal studies, such as following up treated patients where successfully treated cysts become hyperchogenic. (238). Aspiration cytology appears helpful in detecting pulmonary, renal and other non-hepatic lesions for which imaging techniques and serology do not provide appropriate support. Immunodiagnostic tests for the detection of serum antibodies are used to support the clinical diagnosis of E. granulosus infection. (327:328). The indirect

haemagglutination tests and ELISA using E. granulosus hydatid fluid antigen are relatively sensitive for hepatic cases, pulmonary cyst localization, and multiple organs localization with percentage success of (85-98%), (50-60%), and (90-100%) respectively. (238).

The route of transmission of E. granulosus eggs in humans is diverse. E. granulosus eggs are known to adhere to fur of definitive hosts. (238). Close contact with infected dogs creates a risk of infection. (329). Defecation sites of dogs in areas in which E. granulosus is prevalent are high risk, especially for children or any other individuals, who come in contact with the contaminated soil. (238). Secondary contamination of food produce on soil or which has been putatively contaminated by feces of carnivores may be a significant source of infection. (238). Fecal contamination of drinking water may occur in areas where dogs have access to drinking water sources. (238).

Surgery and Puncture-Aspiration-Injection-Respiration (PAIR), sometimes complemented or replaced by chemotherapy, represent the principal treatment. (330). Perioperative chemotherapy with albendazole or mebendazole is indicated for reducing the risk of secondary E. granulosus infection. (238). Treatment should commence at least four days prior to surgery and continue for one or more months. (238). Treatment of non-rectable cysts with albendazole or mebendazole results in cysts disappearance in 30% of cases, another 30-50% of patients demonstrate degeneration of cysts or significant size reduction, and the cysts of between 20% and 40% of patients under chemotherapy show no morphological change. (326).

The prevention of E. granulosus infection focuses primarily on the veterinary aspects to control the intensity and extensity of infection in carnivore and dog populations. (238). This includes regular treatment of dogs with praziquantel and meat inspection to prevent access of dogs to cysts. (331).

Echinococcus multilocularis

The distribution of E. multilocularis is limited to the Northern Hemisphere. It is found in the sub artic regions of Alaska and Canada, Central and Eastern France, Austria, Germany, the Bering Straits, the former Union of Socialist Soviet Republics, the Northern parts of the People's Republic of China and the Northern parts of other countries. (326: 332). E. multilocularis occurs as adult

tapeworms mainly in red and artic foxes (Vulpes spp.), but dogs and cats can also be involved incidentally as definitive hosts. (238).

The disease caused by E. multilocularis is known as Alveolar echinococcosis, and it affects mainly the liver, but legions may also metastacie to the lungs, brain and other organs. (326). E. multilocularis infection is characterized by a hepatic legion consisting of a dispersed, firm, pale tissue, subsegmented by scattered small cysts and vesicles. (238). The lesion may have fecal, non-peripheral zones of calcification, and in humans lesions rarly exhibit the formation of protoscoleces and blood capsules within vesicles. (238). Clinical symptoms are usually non-specific with mild upper quadrant and epigastric pain. (238). Hepatomegaly linked with obstructive jaundice has been reported. (238).

The time between infection and the occurrence of the first symptoms has been estimated to average between five and fifteen years. (333). E. multilocularis infection is primarily diagnosed by imagine procedures. The procedures with greatest diagnostic value are Coprological techniques and Ultrasonography. (334:326). Irregularly dispersed clusters of calcifications on plain abdominal radiographs may give initial clues. (238). For mass screening programs, Ultrasonography is the preferred imaging procedure. (335). MRI does not visualize microcalcifications but reveals intra-hepatic changes or obstructionof the inferior vena cava or portal venous system. (238). Complementary to the imaging procedures is immunodiagnosis, which represents a secondary diagnostic tool that is useful in confirming the nature of the aetiological agent. (326: 336). Serological tests are of limited value for the assessment of the efficacy of treatment and chemotherapy. (337:338:339).

Dogs and cats may play a significant role in the transmission of the parasites to humans due to close contact. (238). Fox and dog fur contaminated with E. multilocularis eggs is considered to be an important health hazard to fox hunters and dog owners and to their family members. (238). Vegetables grown outdoors, forest fruits, and windfall fruit contaminated by fox or dog feces or eggs stripped from fox fur are considered to be the major source of infection for humans. (238).

The treatment of choice for E. multilocularis infection is the radical surgical resection of the entire parasitic lesion from the liver or other affected organs. (238). Excision of the parasitic lesion follows the principles of radical tumor surgery, and concomitant

chemotherapy is recommended in all cases after radical surgery or after non-surgerical procedures. (238:330). Long term chemotherapy is mandatory in inoperable or only partially resectable cases and in all patients after liver transplantation. (328). Liver transplantation has been proposed for a selected group of patients restricted to the liver or secondary liver disease leading to chronic liver failure. (330). The efficacy of treatment programs by fox baiting with praziquantel is under evaluation as a potential control measure. (238). In highly endemic areas, monthly treatment of cats and dogs with praziquantel may significantly reduce infection risk. (238).

Chapter 10

Nematodes

Nematodes are round worms with elongated, cylindrical, unsegmented bodies. Nematodes cause disease in human and the species of major public health importance are Trichinella spp., (T. spiralis, T. native, T. murelli, and T. britovi); Enterobius vermicularis (pinworm), Trichuris trichiura (whipworm), Capillaria philippinensis, Gnathostroma spingerum, Pseudoteranova, and Parastrongylus spp.

Trichinella spp.

Trichinella spp., cause the disease known as Trichinellosis. Of the eight recognized species of Trichinella only four are known to cause disease in humans. (T. spiralis, T. native, T.murelli, T.britovi). Trichinella spp infection occurs in the United States of America, certain of Europe, Asia-Japan, China, Lebanon, and Korea. The majority of human cases in the United States of America are due to T. spiralis and T. murelli. (340:341). Trichinella infection is initiated by ingesting raw or undercooked meats or meat products from infected pigs, wild boars, horses, walruses, dogs, and other domestic or wild mammals harboring the nerve cell larvae complex. (341:238). Larvae are released from muscle tissue by digestive enzymes in the stomach and then locate to the upper two-thirds of the small intestine. (341). The outer cuticular layer becomes partially digested and this enables the parasite to receive environmental cues and to then select an infection site within the small intestine. (342:343:344). The immature parasites penetrate the columnar epithelium at the base of the villus. (341). They live within a row of these cells and are considered intra-multicellular organisms. (345). Parasite and host cell develop in a coordinated fashion. (341). T. spiralis is infective by the 14^{th} day of infection, but the worm continues to grow in size through the 20^{th} day. (346).

The clinical symptoms caused by Trichinella spp in humans depend on the number of infective larvae, the phase of the infection, the immune status of the host (first infection or reinfection), age, gender, and the species of Trichinella. (238:347). There are two main phases for the infection, enteral (affecting the intestines-enteritis), and parenteral (outside the intestines). (347). The first few days of the infection are characterized by

gastroenteritis, associated with diarrhea, abdominal pain, and vomiting. (341). The enteral phase ensues is secretory in nature, transitory, and abates within 10 days after ingestion of infective tissue. (341). The parenteral phase begins approximately one week after infection and may last for several weeks. (341). The symptoms of this phase include fever and myalgia, bilateral periorbital edema, and petechial hemorrhages, which are seen most clearly in the subungual skin, but are observable in the conjunctivae and mucous membranes. (341). Larvae penetrating tissues other than muscles gives rise to more serious sequelae and cardiovascular involvement may lead to myocarditis. (341). Electrocardiographic (ECG) changes can occur during this phase, even in the absence of symptoms. (341). Parasitic invasion of the diaphragm and accessory muscles of respiration result in dyspnea, and neuro-trichinellosis occurs in association with invasion of the central nervous systems. (341). A convalescent phase follows this acute phase, during which time many but not all, nerve cell parasite complexes are destroyed. (341). It must be noted that a great majority of Trichinella infections have either minor or no symptoms and no complications. (348).

The definitive diagnosis depends upon finding the nerve cell parasite complex in muscle biopsy by microscopic examination, serological tests, or by the detection of Trichinella spp DNA by PCR or ELISA. (349:350). Muscle biopsy can be negative, even in the heaviest infections, due to sampling errors. (341). Additionally, the larvae may be at an early stage of their development, making them inconspicuous, even to experience pathologists. (341). Muscle enzymes, such as creatine phosphokinase (CPK) and lactic dehydrogenase (LDH), are released into the circulation causing an increase in their serum levels and serological tests begin to show positive results within two weeks. (341). ELISA can detect antibodies in some patients as early as 12 days after infection.

There are two stages of treatment for trichinella infection and they depend on the time of diagnosis. The early administration of drugs such as mebendazole or albendazole, given within days of infection, decreases the likelihood of larval encystation, but most cases are diagnosed after three days. (351: 337). After the infection is diagnosed, that is, after three days, steroids such as prednisone are administered to relieve muscle pain associated with larval

migration. Treatment with mebendazole or albendazole is still appropriate.

The prevention of Trichinella infection can be achieved through legislation, education and training, food preparation and farming hygiene. Laws should be passed and enforced to ensure that food processors maintain adequate conditions for the safe production of meat and meat products. The public, including hunters, should be educated about the dangers of consuming raw or undercooked meat from pigs, wild boars, horses, other domestic and wild mammals. Food preparation should ensure that all meats mentioned above to be cooked to an internal temperature of 165F (74 centigrade) or more for at least 15 seconds. Uncooked meat should be frozen to (-15Centigrade or 5F). All meat carcuses should be promply and hygienically disposed of.

Enterobius vermicularis (Pinworm)

Enterobius vermicularis is the nematode commonly called the pinworm. Emterobius vermicularis infection is a common infection of children in temperate areas. It is the most common parasite of children in the United States of America. E. vermicularis infection is caused by the ingestion of eggs (ova). After ingestion, the eggs hatch in the intestinal tract, and the young worms migrate from the small intestines to the large intestines where the larvae mature into adult worms and mate. (352). The female worm then moves to the area around the anus, usually at night to deposit her eggs. (352).The eggs are deposited in a sticky gelatinous substance that adheres to the skin. The eggs and gelatinous material cause the itching. Upon drying, the eggs become airborne and settle in the house dust. (353). Eggs can survive outside the body for as long as 3 weeks at normal room temperature. (352).

Eggs can be transferred from the area around the anus of an infected child to clothing, bedding or toys, by fingers to the mouth of another child who swallows them, by contaminated food and drink or self -inoculation by one's own fingers. (352:353). The symptoms of E. vermicularis infection are pronounced anal itching, disrupted sleep, and sometimes nausea, abdominal discomfort and diarrhea. (353). Most children who are infected with E. vermicularis have no symptoms. (352:353).

The diagnosis of Enterobius vermicularis infection is made by finding the worms or eggs. An examination of the child's anus could reveal the whitish worms as they wiggle outside. Additionally, eggs

can be obtained by placing the sticky side of a strip of transparent tape on the child's anus and perform microscopic examination. (352:353). A single dose of mebendazole, albendazole or pyrantel pamoate effectively cures E. vermicularis infection. (352). Many doctors recommend treating the entire family once a family member is diagnosed with the infection.

The preventative measures of greatest importance to control E. vermicularis infection is hygienic in nature. Clothing, bedding, and toys should be washed to remove eggs. The environment should be vaccum to try to eliminate eggs. All food and drink should be covered to prevent contamination from airborne eggs. Since the fecal oral route is a means of transmission of E. vermicularis infection, proper washing of hands is essential after using toilets.

Trichuris trichiura (Whipworm)

The common name for the nematode, Trichuris turchiura, is the whipworm. Trichuris trichiura infections occur mainly in tropical and subtropical regions of the world where there is poor sanitation. Humans are the only host for the infection. Embryonic eggs deposited in the soil are not immediately infective and continue development for 3 to 6 weeks in that habitat. (353). Ingested eggs hatch in the small intestine, where the larvae attach, penetrate the outer wall, and undergo several moults. (353). The mature adults move to the large intestine and gain a hold with their long thin heads, while the thicker tail dangles free in the intestinal lumen. Following sexual maturation and fertilization, the female eventually lays 3.000 to 5,000 eggs daily in to the bowels. (353).

In humans, T. trichiura infection can be contacted by ingesting eggs through the fecal-oral route, contaminated water or agricultural root and leafy vegetables irrigated with contaminated water or grown in contaminated soil. The symptoms of T. trichiura infection may include localized hemorrhage of the bowel, caused by worms burrowing and piercing intestinal mucosa. (353). Heavy infections can cause dysentery, loss of muscle tone, and rectal prolapse, which can prove fatal in children. (353).

The major routes of transmission of the disease in humans are via the fecal-oral route and contaminated food/water. The drugs of choice for treatment of the infection are mebendazole and albendazole. The diagnosis of the infection can be achieved

through microscopic examination of the stool for eggs. The control and prevention of the infection is possible through proper disposal of feces, avoiding the contamination of rivers used for irrigation of crops and drinking purposes.

Capillaria philippinensis

Capillaria philippinensis occurs over a wide geographical area from the Philippines to Egypt, where the infection occurs as small epidemics along the rivers. (238). C. philippinensis infection occurs from eating raw or undercooked fish. The adult nematode lives in the upper intestine where auto-infection is responsible for maintaining infection for many years. A chronic malabsorption syndrome may develop, which has occasionally been reported to be fatal. (238). Monkeys appear to be the definitive host. The drugs of choice for treating the infection are mebendazole and albendazole. Control is through education of the dangers of eating raw or undercooked fish and the possible effects of monkeys. The parasite has been greatly reduced where the practice of eating undercooked fish has been discontinued. (238).

Gnathostroma spingerum

The most reported cases of G. spingerum infection are from Thailand and Japan. Mammalian carnivores such as cats, dogs, tigers and leopards serve as definitive hosts. (238). Fresh water crustaceans serve as the initial intermediate host. When infected crustaceans are ingested by fish or frogs, these species serve as secondary intermediate hosts in which infected L3 larvae develop. (238). Infection in definitive hosts occurs by eating raw or undercooked fresh water fish. A cutaneous or visceral larva migrans condition may arise in humans when infected fish or frog are eaten. Diagnosis in humans may be possible through microscopic examination for larvae in infected superficial tissue. Treatment for superficially occurring larvae may be surgical removal. Treatment with the drugs mebendazole and albendazole may be useful. Education of the dangers of eating raw or undercooked fresh water fish or frog might be the most useful control measure.

Pseudoterranova decipiens

Pseudoterranova decipiens larvae cause the infection in humans known as aniskiosis. P. decipiens is a common nematode parasite of marine mammals, cod, and other sea fish. (238). Human infection is acquired by consuming undercooked or raw fish filets

commonly sold in stores. Clinical manifestations may include “tingling throat syndrome’ or ulceration of the gut. (354). Therapy include surgical removal of larvae, and the drug ivermectin is assumed to exhibit reasonable efficacy. (238).

Parastrongylus spp.

Thousands of human infections occur with the larval stage of the rodent nematode Parastrongylus cantonensis. Human infection occurs by eating infected molluscs or food contaminated by contact with snails. Infection causes eosinophilic meningitis. The parasite has spread in recent yearswith dissemination of one of the intermediate hosts, the giant African land snail (Achatina fulica), which is a popular food item in some countries. (238). Chemotherapy may include the drugs albendazole or ivermectin, but always requires concomitant medication with steroids. (2380. Control could be the education of individuals to exercise caution when eating molluscs or food contaminated by contact with snails.

Chapter 11

Entamoeba Histolytica

Entamoeba histolytica is one of the more serious parasitic infections of humans, affecting an estimated 50 million individuals world-wide. It is an amoeba, meaning a single cell organism that divides by binary fission and can replicate in the intestinal tract, primarily in the large intestine or colon. In the colon the organisms actually bore their way into the bowel wall, and cause ulcerations and severe bloody diarrhea called dysentery, and abdominal pain.

Amoebic dysentery is one of the classical cause of bloody diarrhea seen more in developing countries without clean water supplies. The typical symptoms include bloody diarrhea, abdominal pain, weight loss, fatigue and dehydration. This can be a serious problem and can be fatal especially in infants and children. It can lead to chronic malnutrition and illness in adults. Rarely in the colon wall, an inflammatory process (due to the amoeba called an ameboma), can develop that leads to bowel obstruction that can mimic cancer, Crohn's disease, and other colon disorders. It requires a biopsy for diagnosis, but is a relatively rare complication. Usually the bowel symptoms are primarily related to dysentery. Fever is present in 855 of cases, bloody diarrhea in most, and more chronic symptoms are noted in longer duration cases such as weight loss and fatigue.

The most serious common complications caused by E. histolytica infections are amoebic liver abscesses. When the organism invades the blood stream through the colon wall it often lodges in the liver where it continues to multiply. The liver reacts and creates an inflammatory wall around the organisms, similar to the process for abscess formation with any bacterial infection. The abscess can become very large, but unlike most large bacterial abscesses these can usually be treated with medications and do not require drainage procedures or surgical excision. Rupture of an amoebic liver abscess can lead to shock and death in a high percentage of cases.

The life cycle of E. histolytica exists in two stages, the infective cyst and the trophozoite that cause disease symptoms. Humans are the primary host for E. histolytica. E. histolytica infections are collectively known as amoebasis. The E. histolytica infections can

occur when individuals ingest water and food contaminated with cysts. Cysts are excreted in feces of humans and can survive in soil. Hence infections can also occur from root and leafy vegetables irrigated with contaminated water or grown in contaminated soil.

The diagnosis of E. histolytica infections are performed using blood CP, hemoglobin estimation, stool examination for cysts and trophozoites, radiography, aspiration exploratory, medical ultrasonography, sigmoidoscopy, liver function tests and serological tests. The treatment of E. histolytica infections should involve drugs called amoebicides, which are directed to all sites where the parasite may be present. The amoebicide should be able to act within the intestinal lumen, the intestinal wall, and systemically, particularly the liver. The systemic amoebicidal drugs used to treat E. histolytica infections include emetine, dehydroemetine, chloroquine diphosphate, metronidazole, and tinidazole.

The transmission of E. histolytica infections are via the feceal oral route, contaminated water and food-vegetables and fruits irrigated with contaminated water or grown in contaminated soil.The control procedures should include proper disposal of sewage, use of proper hygiene through education and food preparation.

Conclusion

Foodborne diseases are a widespread and increasing public Health problem in develop and developing countries. (1). Bacteria, viruses, fungi and parasites are the microorganisms that play a significant role in food borne diseases and are an economic and medical challenge to all countries of the world. The use of jet travel and rapid rate of interactions between individuals from all parts of the world make it easier for food borne diseases from endemic regions to be transferred to other regions. It is therefore important for communication and education to be enforced and made readily available to global travelers. In the situations where vaccines can be used to protect or prevent foodborne disease global travelers should be informed of such protection.

The major symptoms of bacterial infections are fever, vomiting and diarrhea, along with abdominal pain. After diagnosis bacterial food borne diseases are treated with antibiotics. Caution must be exercised in situations where individuals are allergic to certain antibiotics. Botulism is a special case of bacterial food borne disease because it is caused by a toxin and is treated with an antitoxin rather than antibiotics. Immunosuppressed individuals, the young, and the elderly are at risk groups for bacterial food borne disease.

In recent years, there has been an increase in the incidence of food borne disease world-wide, with viruses now recognized as a major cause of food borne disease. (73: 74). The viruses implicated in food borne disease are enteric viruses, which are found in the human intestines, excreted in human feces, and transmitted by the fecal oral route. (73). A lot of different viruses are found in the human gut, but not all are recognized as food borne pathogens. (73). The diseases caused by enteric viruses fall into three main categories: gastroenteritis, enterically transmitted hepatitis, and illnesses that can affect other parts of the body such as the eye, the respiratory system, and central nervous system including conjunctivitis, poliomyelitis, meningitis, and encephalitis. (73). The transmission of a virus is dependent not only with its interaction with the host, but on its interaction with the environment outside the host.

Pre-harvest contamination of foods, food handling, delicatessen meats, shell fish and other seafood, pork and wild game, fruits and vegetables, and cross contamination due to water

supplies are all implicated in the outbreaks of viral food borne diseases. With gastroenteritis symptoms such as fever, diarrhea, vomiting and nausea are common. Once diagnosis is achieved the treatment of choice is antiviral drugs rather than antibiotics. Sometimes the immune system is sufficient for recovery from the infections. Disinfection of surfaces and proper hygiene are good preventative measures to control the diseases.

Fungi are a group of eukaryotic organisms that include microorganisms of the genus, Fusarium, Penicillium, Aspergillus, Claviceps, and mushrooms. Fusarium, Penicillium, Aspergillus and Claviceps grow on the normal flora of food (fruits and vegetables), and can cause infection in normal and immunocompromised individuals if contaminated food is ingested. Fungal food borne disease is still in the research stage and it is not enough to understand that there is a known fungal toxin and several species of fungi can produce the same toxin with similar symptoms. (136). Mushrooms are normally eaten by man, but some species of mushrooms are poisonous and can cause infection that leads to death. Penicillium, Fusarium and Aspergillus are also used by man for their beneficial effects.

When fungal foodborne disease occurs the symptoms are normally similar. Generally there is an acute fungal food poisoning incubation period, which is short, with first gastrointestinal symptoms such as abdominal discomfort, nausea, vomiting, abdominal distention, loss of appetite, and occasional diarrhea. Depending on the type of mycotoxin there is the occurrence of damage to the liver, kidney, nervous system and blood system. Once diagnosis is achieved fungal food borne infections are treated with antifungal drugs.

Mycotoxins are chemical compounds produced by some fungi. These are secondary metabolites of biological origin. Despite efforts to control fungal contamination, toxigenic fungi are ubiquitous in nature and occur regularly in world-wide food supplies due to mold infestation of susceptible agricultural products such as cereals, grains, nuts and fruits. (151). Thousands of mycotoxins exist but only a few present significant safety challenges. (151). There are six classes of mycotoxins that are of importance to man both medically and agriculturally. These are categorized as aflatoxins, ochratoxins, citrinin, ergot alkaloids, patulin and fusarium toxins.

Mycotoxins can enter the food chain as a result of fungal infection of crops, either by being eaten directly by humans or being used in livestock food. Mycotoxins greatly resist decomposition or being broken down in digestion, so they remain in the food chain in meat, and poultry products. Temperature treatments such as cooking and freezing do not destroy mycotoxins.

Mycotoxins occur, and exert their toxic effect in extremely small quantities in foodstuffs. Their identification and quantitative assessment thus generally require sophisticated sampling, sample preparation, extraction, and analytical techniques. The methods used are Thin Layer chromatography (TLC), Gas Chromatography (GC), High-Performance Liquid Chromatography (HPLC). And ELISA. (22o:221). PCR is currently used for the detection of microorganisms in foods. Despite the availability of approved methods, variability in sampling and testing procedure presents difficulties for precise determinations. (151). There remains a need for efficient, cost effective sampling and analysis methods that can be used in laboratories. (151). Instead of PCR microbial DNA can be detected in foods using FTIR Infrared Spectroscopy (355). The preparation of food extracts is desirable for the detection of microbial DNA using FTIR Infrared Spectroscopy. It might be possible to detect mycotoxins using FTIR Infrared Spectroscopy of food extracts. A possible extraction procedure that might be useful is the simple one outlined by Sealy and Carrington (356). The proposed methods are cost effective and simple to use once elucidated. It might be possible to use the same extracts for the detection of microbial DNA by FTIR Infrared Spectroscopy.

Although various wild mushrooms contain an assortment of poisons that are definitely fungal metabolites causing noteworthy public health problems in humans, and in such case the distinction is based on the size of the producing fungus and human infection. (161).Mycotoxin exposure is almost always accidental, whereas with mushrooms improper identification and ingestion of the causal mushroom: poisoning is commonly the case. Mushroom poisoning (also known as mycetism) refers to the harmful effects from the ingestion of toxic substances present in a mushroom. The toxins present in a mushroom are secondary metabolites produced in specific biochemical pathways in fungal cells. Mushroom

poisoning is usually the result of ingestion of wild mushrooms after the misidentification of a toxic species with an edible species.

Of the many thousands of mushrooms species in the world, only 32 have been associated with fatalities, and additional 52 have been identified as containing significant toxins. (222). There are eight well studied mushroom toxins, namely, alpha-amanitin, orellanine, muscarine, gyromitrin, coprine, ibotenic acid, psilocybin, and arabitol. Mushroom toxins produce symptoms according to the toxin present and may include gastrointestinal symptoms such as diarrhea, vomiting, headache, nausea and upset stomach. Other medical (psychiatric) symptoms are possible. Symptoms occur after hours, days, or even weeks after ingestion of toxin. These are usually toxin specific. Some mushroom poisoning are almost instantly fatal whereas others are self-limiting or have fatality rates between 50-90% depending on hospital admission.

Parasitic food borne disease always have an intermediate or definitive host before transmission to humans. Some of these parasites in addition to causing food borne disease are also water borne and soil borne. These water borne and soil borne aspects mean that they can cause disease either by direct ingestion of the infected water by humans or by crops grown in contaminated soil or irrigated with contaminated water. A major route for the transmission of parasitic infections is the fecal oral route. Other routes are from contaminated poultry, meat, wild game, seafood, agricultural crops-fruits and vegetables. Transmission via these methods are associated with the consumption of undercooked or raw foodstuffs. Leafy vegetables should be thoroughly washed before consumption. Education pertaining to the dangers of eating raw or undercooked meat/meat products, domestic and wild game, poultry and sea food is important. In poor countries it is important to emphasize that proper sewage disposal is necessary. The use of proper hygiene must be emphasized. Immunocompromised individuals, the young, and the elderly are at high risk for parasitic infections.

The identification of parasites in stool via light microscopy – both modified and simple- is an important technique for diagnosis. However, in the case of cestodes, nematodes, trematodes, and microsporidia where species are determined to be morphologically similar by light microscopy it may be possible to use Scanning Electron Microscopy of prepared slides or paper strips to reveal

morphological differences. In the case of Echinococcus multicularis where the time between infection and the occurrence of the first symptoms has been estimated to average between 5 and 15 years there should be regular monitoring of the at risk groups. A mass screening program should be implemented in the Northern Hemisphere to hunters, pet owners (cats and dogs), and their family, and to red foxes dwelling in the area. The procedures with greatest diagnostic value should be used, namely, Coprological techniques (CT) and Ultrasonography.

All countries, developing and developed should have an agency responsible for monitoring food and food products, recording incidences of food borne disease, educating their population of the importance of proper food preparation and storage, adequate hygiene, and the importance of available vaccines. It must be noted that almost all foods consumed by human can be subjected to microorganisms that can cause food borne disease. Some food borne diseases are very fatal and others are asymptomatic. It is important to note that USDA approved on food and food products does not mean that there is no microbial contamination of food or products, but rather the samples taken showed no evidence of microbial contamination. Additionally, contamination can occur after the USDA inspection.

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