

Biological hazards in foods, Foodborne parasites, Pathogenic flatworms and round worms and Its control measures

Expected Learning Outcome

- Types of biological hazards of human health significance associated with the fish and fishery products.
- Source, characters and problems associated and preventive measures required for combating these hazards.

6.1. Biological hazards

The presence of human pathogenic microorganisms in food has potential human health risk as the consumption of such food could cause food poisoning. Besides bacteria, presence of human pathogenic viruses and several other organisms such as parasites and worms or accumulation of toxic metabolites produced by toxic microalgae in fish and shellfish, and consumption such food could be cause of health related problems. These hazards of biological origin cause a serious health related problems. Hence, there is a need to free the food meant for human consumption form these biological hazards.

6.2. Mycotoxins

- Mycotoxins are toxic substances produced by a large number of molds (fungi), and are highly toxic to animals and potentially toxic to human beings. These are mutagenic, carcinogenic and some show toxicity restricted to specific organs.
- Mycotoxicosis is the syndrome resulting from the ingestion of mold toxin contaminated food. Of the several mold, members of *Penicillium* and *Aspergillus sp* produce mycotoxins as secondary metabolites during the end of exponential growth. Among the mycotoxins, aflatoxin is very important.

6.3. Aflatoxins

Aflatoxins are among the several mycotoxins produced by fungi and are more potent and most widely studied toxin. First reported in 1960 in England, when more than 1 lakh turkey poults died after feeding imported peanut meal from Africa. The causative agent was identified as *Aspengillus flavus* and the toxin was designated as aflatoxin. Aflatoxin is produced by certain stains of *A. flavus* and *A. parasiticus*.

Foods supporting aflatoxin production

These fungi grow in a wide variety of foods/ substances - dairy products, bakery products, fruit juices, cereals, forage crops, dry fish etc. More common in peanuts, cotten seeds and corn as fungal invasion, growth and mycotoxin production takes place before harvesting. Generally, contamination and aflatoxin production is related to insect damage, humidity, weather condition and agriculture practices. These produce aflatoxin in mesophilic temperature range (25-400 C) and water activity of 0.85.

Aflatoxin types

Aflotoxin is composed of 4 major toxic substances (toxic metabolites responsible for aflatoxin) namely B1, G1 and B2 and G2 based on whether they fluoresce blue (B) or green (G) under UV light. *A. flavus* produces B1 and B2 (AFB1 and AFB2) while, *A. parasiticus* produces all four aflatoxins (B1, B2, G1, G2). AFBI is most potent of all aflatoxins.

Fishes fed aflatoxin containing feed (contaminated fish meal and other feed ingredients,

contaminated feed) affects growth and also carcinogenic in trout. International guidelines permit maximum of 30 ppb of aflatoxin in foods and feed ingredients.

6.4. Marine toxins

Marine toxins are natural toxins found in fish and shellfish. Consumption of toxin containing seafood results in various kinds of seafood borne illness. Marine toxins or biotoxins are produced by naturally occurring marine algae / phytoplankton and marine organisms become toxic by feeding on these toxic phytoplankton.

Seafood toxic types

1. Shellfish toxins
2. Ciguatera toxins
3. Tetradon toxins
4. Scombroid toxins

6.4.1. Shellfish toxins

Filter feeding bivalve mollusks become toxic by feeding on blooms of toxic algae (dinoflagellates). Though > 4000 species of marine phytoplankton are known, only about 2% produce toxins. Important shellfish associated illness are,

- Paralytic shellfish poisoning (PSP)
- Diarrhetic shellfish poisoning (DSP)
- Neurotic shellfish poisoning (NSP)
- Amnesic shellfish poisoning (ASP)

Paralytic shellfish poisoning (PSP)

- PSP is caused by eating toxic filter feeding molluscs such as clams, oysters, mussels and cockles.
- Bivalve molluscs become toxic by feeding on certain toxin dinoflagellates which form bloom (> 10⁶ cells/L) and discolor water (red tide).
- The dinoflagellates (toxic) involved are species of *Alexandrium*, *Gonyaulax*, *Gymnodinium*, *Pyrodinium* etc.
- Toxin associated with PSP is saxitoxin which is highly toxic and fatal in small doses (1-4 mg). This toxin is also resistant to heat and acid, and has no antidote. Generally gets destroyed when heated for 3-4 hours at pH 3.
- Symptoms of illness develop within 2 hours after ingestion of toxic molluscs.
- Toxin affects respiratory and cardiovascular regulating center and death occurs due to respiratory failure. Mortality rate is 1-22%.

Symptoms

Paresthesia (tingling, numbness and burning) which begins in mouth, lips, tongue and later spreads over face, scalp and neck, fingertips and toes.

Safe limits

Max allowable limit of PSP toxin in shellfish is 80 µg/ 100 g.

Prevention

- Avoiding eating toxin containing shellfishes.
- Regular monitoring of shellfish growing and harvesting water for toxic algae and toxin levels in shellfish.

- Detoxification of bivalves by natural depuration process or by keeping in clean water.

Diarrhetic shellfish poisoning (DSP)

DSP is caused by eating bivalve molluscs which have accumulated toxin by feeding on bloom of dinoflagellates such as *Dinophysis* and *Prorocentrum*. Toxin responsible for DSP is okadaic acid and its derivatives.

Symptoms

Acute diarrhea with vomiting and abdominal pains. Victims recover within 3-4 days. No mortalities have been reported. Tolerance level is 20 µg/100g.

Neurotoxic shellfish poisoning (NSP)

NSP is caused by consuming shellfish that have been exposed to toxic dinoflagellate bloom of *Ptychodiscus brevis* (*Gymnodinium breve*). Toxin responsible for NSP is a family of brevetoxins which are lipophilic, relatively insoluble in water and soluble in non-aqueous solvents.

Symptoms

Resemble those of PSP except paralysis. It is not fatal and causes neurological symptoms.

Tolerance level: 20 µg /100g (MU: mouse units).

Amnesic shellfish poisoning (ASP)

ASP is caused by consuming bivalve molluscs that have accumulated toxin by feeding on toxic diatom, *Nitzschia*. This is the only shellfish poisoning caused by diatom. Toxin responsible is domoic acid.

Symptoms

Symptoms vary from slight nausea and vomiting to loss of balance, neurological disturbance resulting in confusion and short term memory loss.

Tolerance level (as domoic acid) is 20mg/kg

6.4.2. Ciguatera fish poisoning (CFP)

- CFP is caused from the ingestion of variety of tropical and subtropical carnivorous reef fishes such as Barracuda, Groupers, Seabass, Snappers etc.
- There are over 300 fish species responsible for CFP.
- The herbivorous reef fishes feeding on toxic dinoflagellates (*Gambierdiscus toxicus*) are in turn fed by carnivorous fishes which accumulate toxins in their tissue.
- Toxin accumulation is more in liver followed by viscera and muscle tissue.

Symptoms

- Upon ingestion of toxin containing fishes, symptoms occur within 3-6 hours.
- Symptoms are similar to PSP with gastrointestinal disturbances and neurological disorders. These include vomiting, diarrhea, tingling and burning sensation in mouth, lips and throat, muscle cramping and weakness.
- Death generally occurs due to respiratory failure.
- Levels as low as 1ppb in fish can cause illness.

6.4.3. Tetrodotoxin / pufferfish poisoning

- Puffer fish poisoning is caused due to the consumption of puffer fish (tetradon fish or fugu) which have toxic tissues or organs.
- Pufferfish toxin is thought to be produced by the symbiont bacteria (*Pseudomonas*) associated with the fish.
- Tetrodotoxin is chemically aminoperhydroquinazoline which is similar to saxitoxin of PSP and cause symptoms similar to PSP but of varying degree.
- Only certain species of puffer fish are toxic and toxin is restricted to the skin, liver, viscera, gonads, intestine and muscle.
- Since the fugu is a delicacy in Japan, most cases of intoxication are reported from Japan with instances of death.

Symptoms

Causes neurological symptoms similar to PSP - tingling in lips and extremities, paralysis and death by respiratory arrest and/ or cardiovascular collapse. Mortality rate is high. Cardiovascular effects are more severe than PSP with high death rates.

6.4.4. Scombroid poisoning

Scombroid poisoning or histamine poisoning is caused by the consumption of fishes containing high levels of histamine. Scombroid fishes (tuna, seer fishes, mackerel) containing red meat are implicated in scombroid poisoning. Other fishes like carangids, herrings, sardines and anchovies are also involved in histamine poisoning.

Source of histamine

Scombroid fishes have high levels of histidine which is converted to histamine by the growth of microorganisms possessing the enzyme histidine decarboxylase. Conversion of histidine to histamine by microorganisms results in the accumulation of histamine in fish.

Bacteria involved in decarboxylation are *Morganella morgani*, *Klebsiella pneumoniae*, *Enterobacter aerogenes* and *Haffnia alvei*. The main source of these bacteria to fish is from post harvest contamination. Though these bacteria grow well at 10°C, highest histamine production occurs at 37°C. Histamine is heat resistant.

Symptoms

- Symptoms of poisoning occur with short incubation period (few minutes to few hours), the illness is mild and self limiting lasting for only few hours.
- Gastrointestinal disturbance (nausea, vomiting, diarrhea), facial flushing, labial edema, itching of the skin and rashes on skin are the common symptoms of illness.
- Maximum permissible limit in seafood is 50 ppm.

6.5. Foodborne parasites

Several human parasites are associated with fish and shellfish and consumption of such foods leads to transmission of parasites to humans. These include members belonging to the group protozoans, flat worms and round worms. These parasites are easily detectable in food because of their larger size than bacteria, unable to proliferate in food, not possible to grow on culture media, and complete life cycle by passing through one or more animal hosts. Consumption of foods contain these parasites could lead to health risks.

6.5.1. Protozoan parasites

The important protozoan parasites involved in human illness are members of the genus *Giardia*, *Entamoeba*, *Toxoplasma*, *Sarcocystis* and *Cryptosporidium*.

Giardia Sp

The flagellated protozoan *Giardia lamblia* exists naturally in aquatic environment and causes

human illness called giardiasis.

Characters

- The *Giardia* cells (trophozoites) produce cysts which infect humans through water and food. *Trophozoites* are characterized by the presence of eight flagella arising on the ventral surface near the paired nuclei.
- The cysts are pear shaped, with the size ranging from 8-20 μm in length and 5-12 μm in width.
- The excystation of ingested cysts in upper small intestine releases the trophozoites which invade intestinal wall and bile duct.
- Growth is generally favoured by high levels of bile juice in the duodenum and upper jejunum.

Symptoms of illness

- Giardiasis is highly contagious.
- The disease symptoms appear after a incubation period of 6-13 days, cysts appear in stools in 3-4 weeks, and symptoms may last for months to a year or more.
- The leading symptoms of infection are diarrhea, fatigue, abdominal cramps, fever, nausea, vomiting and weight loss.
- The minimum dose required for infection in humans is 10 cysts or less.

Control measures

- Avoiding sewage contamination of natural waters.
- Avoiding consumption of sewage contaminated water and fish harvested from sewage polluted waters.

Entamoeba histolytica

This protozoan parasite is responsible for amebiasis or amoebic dysentery and is transmitted mainly through fecal-oral route, and also through water, food handlers and foods.

Characters

- *Entamoeba histolytica* is an motile aerotolerant anaerobe, trophozoites are of 10-60 μm in size and lack mitochondria, cysts non motile and are of 10-20 μm in size.
- Trophozoites do not persist in the environment but cysts can survive in sewage sludge for up to 3 months and transmitted through water and food.
- The trophozoites in intestine adhere to host cell glycoprotein, cause abscesses in intestinal mucosal cells and ulcers in colon, multiply by binary fission in large intestine, encysts in ileum, and produce enterotoxin protein.

Disease symptoms

- After ingestion of the parasite
- The symptoms of disease occur after 2-4 weeks of incubation of ingested parasite and symptoms persist for several months.
- The common symptoms include mucus and blood in stool, abdominal pain, fever, severe diarrhea, and vomiting and weight loss.

- Affected persons can be treated using amebicidal drugs such as metronidazole and chloroquine.

Toxoplasma gondii

The obligate intracellular protozoan, *Toxoplasma gondii*, is responsible for causing a disease called toxoplasmosis.

Characters

- Domestic and wild cats are the definitive hosts for this parasite and serve as primary source of human infection.
- Oocysts are transmitted from cat to cat and infect all other animals, and survive over a year in warm, moist environment.
- Clinical symptoms in humans are caused by as few as 100 oocytes. Encysted form can remain latent in humans.

Disease symptoms

- Toxoplasmosis is regarded as universal infection and symptomless in most individuals.
- When severe, symptoms of fever with rash, headache, muscle ache and pain and swelling of lymph nodes is noticed.

Control measures

- Avoiding environmental contamination with cat feces.
- Avoiding consumption of meat and meat products containing viable tissue cysts.
- Heating food above 60°C which destroys cysts.

Sarcocystis sp

- *Sarcocystis sp* cause sarcocystosis in humans.
- Cattle and pigs serve as intermediate host while humans are definitive hosts.
- Species involved in disease include *Sarcocystis hominis* which is associated with cattle, and *Sarcocystis suis hominis*, associated with pigs.
- Infection occurs through consumption of infected porcine and bovine meat, contaminated food and water.
- Sarcocysts in humans transform to bradyzoites which penetrate small intestine, reproduce sexually resulting in sporocysts and pass out through feces.
- The sporocysts when ingested by pigs or bovines release sporozoites and spread throughout the body.
- These multiply asexually in skeletal and cardiac muscle producing sarcocysts.
- Sarcocysts are visible to naked eye as they reach a size of about 1cm.

Disease symptoms

- Symptoms occur within 3-6 hr of infection and consist of nausea, stomachache, and diarrhea.

6.5.2. Pathogenic flatworms and round worms

Occurrence of flatworms and round worms is common in fish and shellfish and over 50 species of helminth parasites from fish and shellfish are known to cause disease in human beings. These parasitic helminths have complicated life cycles during their development involving a number of intermediate hosts. Generally, sea snails or crustaceans serve as first intermediate host, marine fish as second intermediate host, and mammals as the final host for sexually mature parasites. In between these hosts, one or more free living stages may occur. Infection of human may be part of this life cycle or it may be a side track causing disruption of the life cycle.

The pathogenic flatworms and round worms transmitted by fish and shellfish are

Nematodes or Round worms		Fish/shellfish involved
<i>Anisakis simplex</i>		Herring
<i>Pseudoterranova dicipiens</i>		Cod
<i>Gnathostoma sp.</i>		Fresh water fish, frog
<i>Capillaria sp.</i>		Freshwater fish
<i>Angiostrongylus sp.</i>		Freshwater prawns, snails, fish
Cestodes or tapeworms		
<i>Diphyllobothrium latum</i>		Freshwater fish
<i>D. pacificum</i>		Marine fish
Trematodes or flukes		
<i>Clonorchis sp.</i>		Freshwater fish, snails
<i>Opisthorchis sp.</i>		Freshwater fish <i>Paragonimus sp.</i> Snails, Crustaceans, Fishes
<i>Echinostoma sp.</i>		Clams, Freshwater fishes, Snails
<i>Heterophyes sp.</i>		Snails, Freshwater fish, Brackishwater fish
<i>Metagonimus yokagawai</i>		Freshwater fish

A. Nematodes or round worms

- Round worms or nematodes are common and found in marine fish all over the world.
- *Anisakis simplex* is commonly known as "herring worm" and *Pseudoterranova dicipiens* is known as "Cod worm".
- Live worms when ingested by humans penetrate into the wall of the gastrointestinal tract and cause an acute inflammation ("herring worm disease").
- *Gnathostoma sp* is another nematode found in freshwater fish in Asia. The ingested larvae migrate from the stomach to other body regions (subcutaneous sites in the thorax, arms, head and neck) and induce a creeping sensation and edema.
- *Capillaria philippinensis* infection in humans causes severe diarrhea and possible death due to fluid loss.
- *Angiostrongylus cantonensis* is a common nematode in Asia and is associated with freshwater fish, snails and prawns, and known to cause meningitis in humans.

B. Cestodes

- Very few cestodes or tapeworms are transmitted to humans through fish.
- The broad fish tapeworm, *Diphyllobothrium latum* is a common human parasite affecting the intestinal tract of humans.
- The related species (*D. pacificum*) is transmitted by marine fish.
- These parasites are transmitted through the consumption of raw or semi processed fish.

C. Trematodes

- Some of the trematodes or flukes are common in Asia. The liver fluke (*Clonorchis sinensis*) is a common parasite infecting bile-ducts in the liver of humans in Asia.
- Flukes such as *Metagonimus yokogawai* and *Heterophyes heterophies* infect the intestines of the final host (humans) causing inflammation, symptoms of diarrhea and abdominal pain. The adult oriental lung fluke, *Paragonimus sp.* lives in cysts in the lungs of final hosts.

6.5.3. Control of parasitic infection

All infections involving parasites are transmitted by eating raw or uncooked fish products. Control measures to reduce the public health problem related to the presence of parasites include:

- Avoidance of capture and consumption of nematode-infected fish by selecting specific fishing grounds, specific species or specific age groups.
- Sorting and removal of nematode-infected fish or removal of nematodes from fish.
- Application of techniques to kill nematodes in the fish flesh (Ex. Freezing)

6.6. Foodborne viruses

The incidence of foodborne outbreaks of viral gastroenteritis is quite common. Viral disease transmission to human beings via consumption of seafood has been known since the 1950s and human enteric viruses are implicated as a major cause of shellfish-associated disease conditions. Presently, more than 100 known enteric viruses are excreted along with faeces by infected individuals and finally find their way in to domestic sewage.

The seafood associated viral infection causing illness are ;

- Hepatitis- typeA (HAV)
- Norwalk virus (small, round structured)
- Snow Mountain agent
- Calcivirus
- Astrovirus
- Non-A and Non-B viruses

Their presence pathogenic viruses in seafood are mainly due to the contamination through infected food handlers and sewage polluted water. Shellfish being filter feeders filter large volume of water (upto 1,500 litres/day/oyster) and concentrate virus from the water. The concentration of virus in the shellfish is much higher than in the surrounding water. The infection with a few number of viral particles can cause food-borne disease.

Infected persons discharge large quantities of virus in the feces and contamination of food occurs by direct or indirect contact with faecal matter. Among the foods the outbreaks of viral diseases is dominated by bivalve molluscs. Infected food handlers are involved in the transmission of the virus and any food that comes into contact with human hands and does not subsequently receive a substantial heat treatment get contaminated by viruses.

Survival of viruses in the environment and in food

- The survival of viruses in the environment and in food is dependent on factors such as temperature, salinity, solar radiation, and presence of organic solids. Enteric viruses survive much longer than coliform bacteria, and for several months in seawater at temperatures <math><10^{\circ}\text{C}</math>. Thus, there is little or no correlation between presence of virus and coliforms which are the common indicator bacteria for faecal pollution. All enteric viruses are resistant to acid pH, proteolytic enzymes and bile salts in the gut.
- Hepatitis type A virus is one of the more heat stable viruses and has an inactivation time of 10 min. at - Enteric viruses are resistant to some commonly used disinfectants (e.g. phenolics, ethanol, quaternary ammonium compounds), but sensitive to halogens (e.g. chlorine, iodine). Ozone is highly effective in clean water.

Foods involved in viral infection

Seafood-associated viral infections are mainly due to the consumption of raw or improperly cooked molluscan shellfish. HAV transmission has been attributed to unsanitary practices during processing, distribution or food handling. One of the largest outbreaks of food-borne illnesses involving 2, 90,000 cases was reported in China in 1988 due to the consumption of contaminated and inadequately cooked clams.

Prevention of foodborne viral disease

- Prevention of foodborne viral disease mainly relies on measures taken to prevent direct or indirect faecal contamination of food that will not receive a virucidal treatment before consumption.
- Consumption of bivalve shellfish harvested from pollution-free waters or depurated in clean seawater or by cooked.
- Preventing contamination by food handlers by adopting good personal hygiene and health education.
- Preventing food handlers suffering from intestinal infections from handling foods.