Foodborne Viral Pathogens and Infective Protein

6

Introduction

Foodborne viral diseases account for about 5.5 million illnesses, 15,284 hospitalizations, and 156 deaths each year in the United States of America. Estimated economic burden for foodborne viruses is about 3 billion dollars. Viruses are obligate intracellular parasites, requiring a live host for replication. They cannot grow outside their specific host or within foods. Viruses are generally "host-specific," i.e., infecting specific plants, animal, human, or bacteria and usually do not establish cross-species infections. However, zoonotic viruses are able to infect humans, and other viruses can occasionally undergo genetic modifications to adapt themselves to different hosts.

The majority of foodborne viruses are considered enteric due to their fecal–oral mode of transmission. Enteric viruses are generally nonenveloped virus and are stable in the environment. There are four acute gastroenteritis-causing viruses: *Calicivirus*, *Rotavirus*, *Astrovirus*, and *Adenovirus*. *Norovirus* (*Calicivirus*) causes about 5.4 million illnesses in the USA per annum, while *Rotavirus*, *Astrovirus*, and *Sapovirus* cause approximately 15,000 cases, and hepatitis A virus causes approximately 1500 cases.

Enteric viruses (Table 6.1) are highly infectious. A low dose of virus consisting of as few as 10–100 particles is sufficient to cause foodborne infection. Virus life cycle consists of several steps including: (1) attachment to host receptor, (2) penetration of the host cells, (3) uncoating of RNA/DNA, (4) transcription and/or translation, (5) RNA/DNA replication, (6) assembly and packaging of nucleic acid with viral proteins, and (7) release of matured virus particles (see Chap. 2). RNA virus encodes genes for RNA-dependent RNA polymerase, a nonstructural enzyme (replicase), which is needed for RNA replication inside the host, while the DNA-dependent RNA polymerase (called RNA transcriptase) is required for transcription of RNA from DNA and protein synthesis such as capsid protein for viral packaging. Viruses do not carry any genes for metabolism and hence rely on host cells for propagation.

Sources and Transmission

Contaminated foods readily transmit the virus. Primary contamination occurs before harvesting. Examples include oyster and clams (which concentrate virus particles) and vegetables that are irrigated with contaminated or polluted water. Secondary transmission occurs during processing or handling of products where fecally contaminated hands are in contact with foods. Secondary transmission can also occur when vomitus, containing virus particles, is aerosolized, contaminating foods and food contact surfaces. Uncooked foods that receive no heat treatment are common sources of virus infection.

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Table 6.1 Foodborne viruses

Name	Family (Genus)	Size (genome)	Foodborne	Incubation period in days (median)
Poliovirus, Coxsackievirus, Echovirus, Enterovirus	*Picornaviridae* (*Enterovirus*)	28 nm (ssRNA)	Yes, mainly water, present in shellfish	1–5 (3)
Astrovirus	*Astroviridae*	28 nm (ssRNA)	Yes, shellfish	2–3
Hepatitis A virus	*Picornaviridae* (*Hepatovirus*)	28 nm (ssRNA)	Yes	15–50 (28)
Hepatitis E virus	*Hepeviridae* (*Hepevirus*)	34 nm (ssRNA)	Mainly water	14–60
Rotavirus	*Reoviridae* (*Rotavirus*)	70 nm (dsRNA)	Rare often water	1–4 (2)
Sapovirus	*Caliciviridae* (*Sapovirus*)	34 nm (ssRNA)	Yes (rare), mainly shellfish	1–3 (2)
Adenovirus group F, serotypes 40 and 41	*Adenoviridae* (*Mastadenovirus*)	100 nm (dsDNA)	Water, shellfish	3–10 (5)
Norovirus	*Caliciviridae* (*Norovirus*)	34 nm (ssRNA)	Yes	1–2 (1)
Nipah virus	*Paramyxoviridae* (*Henipavirus*)	500 nm (ssRNA)	Fruit bat, fruits, and sap	7–14
Ebola virus	*Filoviridae* (*Ebolavirus*)	1200 nm (ssRNA)	Yes, Fruit bat and other animals	2–21 (7–10)

These foods include salads, bakery products, and raw shellfish. Ice made from virus-contaminated water can also be a source of transmission.

Virus Classification/Taxonomy

Enteric viruses are generally nonenveloped viruses and belong to the family of *Adenoviridae*, *Caliciviridae*, *Hepeviridae*, *Picornaviridae*, and *Reoviridae*. Viruses are classified based on the size, shape, structure, and nucleic acid content (Fig. 6.1). Viruses contain either DNA or RNA with single- or double-stranded nucleic acid molecules. Adenovirus has double-stranded DNA, and RNA viruses generally have single-stranded RNA molecules. Based on the genetic elements, viruses can be classified into seven types: dsDNA, ssDNA, dsRNA, (+) sense ssRNA, (−) sense ssRNA, RNA reverse-transcribing viruses, and DNA reverse-transcribing viruses.

Foodborne Viral Pathogens

Adenovirus

Adenovirus has a large icosahedral structure containing a double-stranded DNA (genome 28–45 kb long). Adenovirus is a nonenveloped virus. It is a member of *Adenoviridae* family and genus *Mastadenovirus*, which includes >20 known viruses (5 human, 3 bovine and 3 porcine, and 9 other species). There are 51 serotypes of human adenovirus. While many adenoviruses can infect the intestinal tract, serotypes 40 and 41 cause the majority of human adeno-gastroenteritis and are shed in feces in large numbers. The incubation period is about 3–10 days and the illness lasts for about a week. Immunocompetent adults are more resistant to these viruses, while children below 2 years of age are most susceptible. The clinical symptoms are associated with gastrointestinal complications involving watery diarrhea. Waterborne outbreak resulting in conjunctivitis has been reported. Failure in proper chlorination of water may lead to an outbreak. Adenovirus is also frequently found in shellfish.

Astrovirus

Astroviruses are RNA viruses and are small (28 nm diameter) with star-like surface projections. Human astroviruses have eight serotypes, with serotypes 1 and 2 being predominant in children. It causes diarrhea in children and the illness is generally mild. The incubation period is

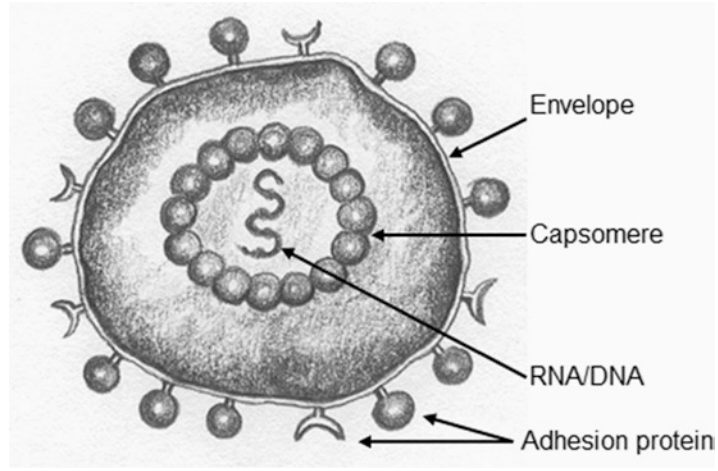

Fig. 6.1 Schematics of a model virus showing various structural components

2–3 days and the disease lasts for about 3–4 days. A major foodborne outbreak was reported in Japan in 1994 affecting 1500 school children and the teachers. Serotype 4 was responsible for that outbreak. The virus can be cultured using mammalian cells.

Rotavirus

Rotavirus looks like a wheel (rota means a "wheel") in which capsid proteins are arranged like spokes of a wheel. The capsid structure consists of an inner core, an intermediate capsid, and an outer capsid with short radiating spikes (Fig. 6.2). It is a large dsRNA nonenveloped virus belonging to *Reoviridae* family. The segmented genome encodes six structural viral proteins (VP1–VP4, VP6, and VP7) and six nonstructural proteins (NSP1–NSP4, NSP5/NSP6). Among the VP proteins, VP1 is the RNA-dependent RNA polymerase, VP2 is core protein, VP3 is methyltransferase, VP6 is inner capsid, and VP4 and VP7 are outer capsids. Among the NSPs, NSP1 is an interferon antagonist, and NSP4 is an enterotoxin. Rotavirus is grouped in eight (A–H) groups. Groups A, B, C, and H are known to infect humans. Of which, group A is responsible for more than 90% of all infections in humans.

Rotavirus primarily infects children of less than 5 years of age and causes acute gastroenteritis.

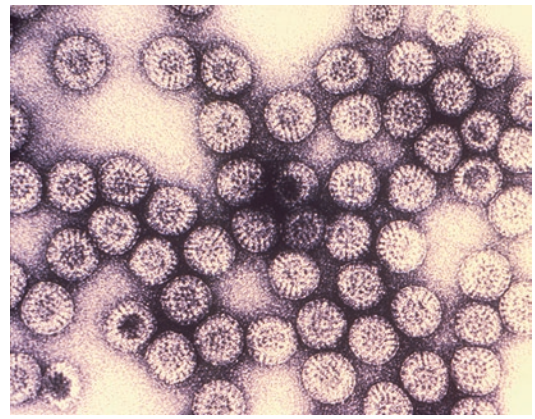

Fig. 6.2 Electron microscopic picture of rotaviruses (70 nm particles). Bar = 100 nm (Courtesy of Dr. Erskine L. Palmer, CDC, Atlanta, GA)

Foodborne rotavirus is responsible for an estimated 15,000 cases per year in the USA worldwide; rotavirus causes about 215,000 deaths annually in children under age 5. The incubation period is about 1–4 days, and the illness is manifested by diarrhea, vomiting, and fever, lasting for a week. Severe dehydration and electrolyte imbalance are responsible for fatalities. A cell culture model (MA104, monkey kidney epithelial cell line) is available to study rotavirus pathogenesis.

Rotavirus infection starts with viral attachment to matured enterocytes using VP4 to the host cell glycan (i.e., sialic acid). The virus then enters the enterocytes at the tip of the small intestinal villi. Histo-blood group antigens

(HBGAs) may serve as a receptor for some rotaviral strains. Virus induces structural changes such as villous atrophy and infiltration of mononuclear inflammatory cells in the lamina propria. Rotaviruses are released from infected epithelial cells without destroying them or causing cell death. Thus, maldigestion and maladsorption of nutrients and a consequent inhibition of reabsorption of water lead to diarrhea. Nonstructural viral protein, NSP4, has been shown to act as an enterotoxin. It promotes chloride secretion and fluid loss. Chloride secretion response is regulated by phospholipase C-dependent calcium signaling pathway. NSP4 may disrupt tight junction barrier function and may induce paracellular permeability. Viruses are released in the stool in high numbers (about 10^9 particles per gram) and can contribute to the fecal–oral transmission. Diagnosis is relatively simple which is accomplished by electron microscopy, agglutination assay or enzyme-linked immunosorbent assay (ELISA), and reverse transcriptase PCR with stool specimen. Rotavirus infection can induce a long-lasting immunity. Two live, attenuated oral vaccines, Rotarix® and RotaTeq® are available to prevent rotavirus-associated gastroenteritis and mortality.

Hepatitis Viruses

Hepatitis viruses are a major public health concern worldwide. They are grouped into hepatitis A, B, C, D, and E (Table 6.2). Hepatitis A virus (HAV) and hepatitis E virus (HEV) are known food–/waterborne pathogens, while hepatitis B (HBV), C (HCV), and D (HDV) viruses are blood-borne and are responsible for acute and chronic liver diseases. A brief description of HBV, HCV, and HDV is included to have some understanding of non-foodborne hepatitis viruses, while HAV and HEV as food–/waterborne pathogens are discussed in detail below.

Hepatitis B Virus

Hepatitis B virus (HBV) is responsible for about 786,000 deaths per year globally. It is a double-stranded DNA virus (3.2 kb) and is transmitted through contact with infected blood and semen. It causes sexually transmitted disease with a high rate of infection seen in homosexuals or heterosexuals with multiple sexual partners, injection drug users, and healthcare personnel. Perinatal infection from mother to neonates is common in the high endemic area. It causes chronic infection. Patients suffer from liver cirrhosis or hepatic carcinoma. A prophylactic vaccine made from recombinant DNA that expresses hepatitis B virus surface antigen (HBsAg) is highly effective.

Hepatitis C Virus

Hepatitis C virus (HCV) is a single-stranded RNA virus with 9.6 kb genome. It exhibits a high rate of mutation due to the lack of proofreading activities of RNA-dependent RNA polymerase. It belongs to *Flaviviridae* family. It infects about 130–170 million people worldwide, and these patients are in the high risk of developing hepatosteatosis (accumulation of lipids in the liver), liver fibrosis (scarring), liver cirrhosis, and hepatic cancer. Transmission occurs predomi-

Table 6.2 Characteristics of hepatitis viruses

Hepatitis virus	Genome	Properties	Clinical significance
HAV	ssRNA(−); 7.5 kb; 27–32 nm	Fecal–oral transmission	Hepatitis, jaundice
HBV	dsDNA; 3.2 kb	Blood-borne	Acute and chronic liver disease
HCV	ssRNA(+); 9.6 kb	Blood-borne	Acute and chronic liver disease; hepatosteatosis (accumulation of lipids in the liver), liver fibrosis (scarring), liver cirrhosis, and hepatic cancer
HDV	ssRNA; 1.7 kb	Blood-borne	Coinfection with HBV
HEV	ssRNA(+); 7.5 kb; 27–34 nm	Fecal–oral transmission; water	Acute hepatitis; increased mortality during pregnancy

nantly through contaminated blood due to blood transfusion, intravenous drugs, organ transplants, and vertical transmission from mother to child.

Hepatitis D Virus

Hepatitis D Virus (HDV) is also known as "delta hepatitis" infection and is often associated with HBV infection. HDV contains a small RNA genome (1.7 kb) with single ORF (open reading frame) and is unable to synthesize its own envelope protein to form a fully functional virion. Thus, it is called defective virus particle or satellite virus and depends on HBV to supply the envelope protein or surface antigens (HBsAgs) for packaging during coinfection with HBV. HDV infection is blood-borne, and infection can spread during coinfection with HBV. HDV infection is involved in acute or chronic liver disease and infection is uncommon in the USA. Globally more than 15–20 million people are infected by HDV.

Hepatitis A Virus

Introduction

The hepatitis A virus (HAV) was identified in 1972 when an immunoelectron microscopy was used for diagnosis. The most common infection routes are person-to-person (fecal–oral route) via household contact, or in homosexual men, intravenous drug users sharing the same needles, and exposure to contaminated food and water. Foods implicated in outbreaks including clams, mussels, raw oysters, lettuce, ice slush beverages, frozen strawberries, blueberries, raspberries, and green onions. The largest outbreak occurred in 1988 in Shanghai (China) due to consumption of contaminated clams, and about 300,000 people showed the symptom of acute hepatitis with 47 deaths. Generally, the HAV infection is asymptomatic in children under 6 years of age, while it is symptomatic in older children and adults with jaundice occurring in greater than 70% patients. Annually, approximately 1.4 million people suffer from HAV infection worldwide.

Biology

HAV particle is 27–32 nm in diameter and has icosahedral symmetry. It is a nonenveloped single-stranded RNA virus (7.5 kb) and belongs to *Picornaviridae* family and genus, *Hepatovirus*. HAV has seven genotypes and four of them (IA, IB, II, III) are associated with human infection and three others (IV, V, and VI) are associated with nonhuman primates. The viral genome encodes a single open reading frame for a polyprotein of 250 kDa. The polyprotein has three regions: P1 represents structural protein, i.e., the capsid protein, and P2 and P3 represent nonstructural proteins required for RNA synthesis and virion assembly.

Pathogenesis

The incubation period of the disease is about 15–50 days (median 28 days). From the intestine, HAV reaches to the liver after a systemic circulation. It interacts with the host cell receptor protein called HAVCR1 (hepatitis A virus cell receptor 1) before entry into hepatocytes. It replicates inside hepatocytes, moves to the gall bladder and released into bile, and eventually is shed in the stool. Infection results in inflammation in the liver. Viruses impair liver function and as a result, bilirubin accumulates in blood and jaundice develops. Two to three weeks after the infection, the immune response to virus develops. Consequently, activated immune cells, $CD8^+$ T, and NK cells attack virus-infected hepatocytes to eliminate the virally infected cells. As a result, hepatocytes are severely damaged manifesting characteristic viral pathogenesis.

The major symptom of hepatitis is jaundice, characterized by yellow discoloration of the skin and the white part of the eye. In jaundice patient, the stool is pale colored and the urine becomes dark. Anorexia, vomiting, malaise, and fever are manifested in the hepatitis patients, and virus particles are shed in large numbers in the stool (about 10^9 particles per gram). Viruses are also shed through saliva. Liver failure may occur in patients with the underlying chronic liver disease. Children may exhibit asymptomatic HAV infection and shed viruses longer than the adults do, while older children and adults show symptoms. The long-lasting immunity primarily humoral (antibody) response, after primary infection, is seen in patients.

Immune Response

The innate immunity involves the production of interferons (IFNα, IFNβ) and antiviral state of viral recognition through pathogen recognition receptors (PRRs) or pathogen-associated molecular patterns (PAMPs). The adaptive response includes the production of IFNγ and activation of $CD4^+$ regulatory T cells (Treg) that express $CD25^+$ and FoxP3 and suppress the immune response to the pathogen. Cytotoxic $CD8^+$ T cells respond to viral antigens through MHC class I pathway. HAV also induces a long-term humoral antibody response.

Prevention and Control

Detection of HAV is achieved by immunological methods such as immunofluorescence assay, dot blot assay, immunoblotting, and ELISA. In the cell culture using African green monkey kidney cell line (Vero) and the fetal rhesus monkey kidney cell, virus replication could be detected in 2–4 weeks by immunoassays. Reverse transcriptase-polymerase chain reaction (RT-PCR) and real-time PCR assays have been used to detect the virus. More recently, nucleic acid sequencing of the PCR-amplified products has been done to confirm and to determine the genetic relatedness among the HAV isolates.

It is difficult to trace the food source because of the long incubation period of the disease. HAV may survive about a month in the environment. It is resistant to chlorine and requires 1 min exposure to 1:100 dilutions of household bleach (sodium hypochlorite). Inactivation by heating requires >85 °C for 1 min. Immunization has been effective in reducing the hepatitis cases, especially in children. The reduction in children hepatitis cases possibly affects the hepatitis infection cycle thus probably reduces the number of adult hepatitis cases in recent years. However, total numbers of sporadic hepatitis cases have not been reduced. Vaccination of food handlers may reduce HAV cases but may not be cost-effective. Personal hygiene and hand washing after toilet visit are the most effective practice in preventing the transmission of HAV.

Hepatitis E Virus

Introduction

Originally, identified as an "atypical hepatitis A virus" that caused an outbreak in New Delhi (India) and infected about 29,000 people in 1955, hepatitis E virus (HEV) was originally classified as hepatitis non-A, non-B virus. Now it has been renamed hepatitis E virus. This virus causes acute hepatitis, annually infecting about 20 million people worldwide. HEV is a member of family *Hepeviridae*, genus *Hepevirus*, and there are four genotypes. Genotype 1 and 2 are associated with person-to-person human infection, and genotype 3 and 4 are associated with zoonotic transmission to humans, from pigs and other species.

HEV is primarily a waterborne virus and caused several outbreaks in developing countries in Asia, Latin America, and Africa. Swine serves as the most important reservoir for HEV. HEV infection can also transfer through organ transplants. The high-risk group includes patients suffering from chronic liver disease and pregnant women and young children. Adults and young adults are also susceptible. Though it is a waterborne virus, several recent outbreaks were associated with consumption of deer meat and raw or undercooked swine liver in Japan. HEV has been routinely isolated from swine from Canada, South Korea, Japan, Spain, the Netherlands, New Zealand, and the USA implying potential future outbreak of HEV with the consumption of undercooked pork meat.

Biology

HEV is a nonenveloped icosahedral-shaped spherical particle (27–34 nm) containing a single-stranded RNA with genome size, 7.2 kb. The genome contains three ORFs: ORF1 encodes nonstructural proteins, RNA-dependent RNA polymerase (RdRP), protease, methyltransferase, and helicase; ORF2 encodes a structural protein, capsid that is required for viral entry; and ORF3 encodes phosphorylated protein required for viral release from the host cells.

Pathogenesis

The incubation period of HEV varies from 2 weeks to 2 months. Propagation of human HEV

is currently challenging but hepatic cell lines, Huh7, HepG2, lung cell line A549, and colon cell line Caco-2, have been used as cell culture models to study pathogenesis. Pathogenesis involves several steps that require viral interaction with the host cell receptor, heparin sulfate proteoglycans, and to another uncharacterized receptor molecule. Upon internalization, viral uncoating allows RNA release followed by RNA replication, packaging, and release of mature virions from the host cells.

The disease is dose dependent: higher dose shows clinical symptoms while lower dose exhibits subclinical infection. It is responsible for acute viral hepatitis similar to HAV and manifests mild jaundice, anorexia, and hepatomegaly. Some patients suffer from abdominal pain, nausea, vomiting, and fever.

Norovirus

Introduction

Norovirus (formerly Norwalk virus) is the leading cause of gastroenteritis and a major public health concern worldwide. The first outbreak occurred in 1968 in children and the adults in Norwalk, OH (USA), hence the name Norwalk, but the virus was not identified until 1972. Dr. Albert Z. Kapikian (1930–2014) at the National Institutes of Health was the first person to identify the virus using an electron microscopy (Fig. 6.3). The Norwalk virus is now called Norovirus (NoV). NoV is highly contagious and the transmission routes are food, water, person, and environment (Fig. 6.4). About 20 million Americans are affected every year resulting in 56,000–71,000 hospitalizations and 570–800 deaths. Of the total illnesses, about 5.5 million illnesses are attributed to food.

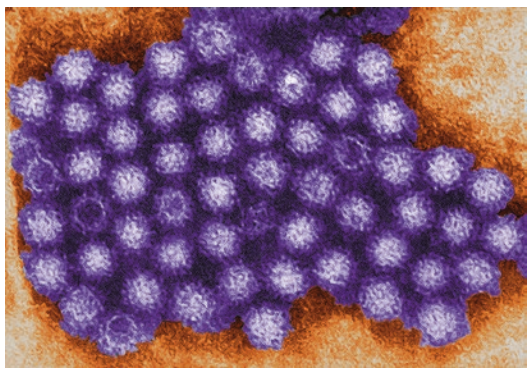

Fig. 6.3 Electron microscopic image of norovirus (Courtesy of Charles D. Humphrey, CDC, Atlanta, GA)

NoV has been responsible for numerous outbreaks in various establishments: the cruise ships, restaurants, swimming pool, schools, nursing homes, and hospitals. Primary transmission to humans can happen through food, and then secondary transmission occurs from fecal–oral or person-to-person and from the environment. Secondary transmissions are expedited when people are in close contact in settings like hospitals, cruise ships, hotels, restaurants, nursing homes, day-care centers, prisons, military installments, and sports stadiums. Fresh produce such as lettuce, tomato, melons, green onions, strawberries, raspberries, peppers, fresh-cut fruits, salads, and food handlers, processors, and irrigation water are also involved in the transmission. Transmission can occur through filter-feeding bivalves including muscles, oysters, and clams, which collect viruses in their tissues.

Biology

Norovirus is also known as a small round structured virus (SRSV) of 27–38 nm diameters with icosahedral shape (Fig. 6.3). It is a nonenveloped virus carrying a plus-sense single-stranded RNA, and the 7.4–7.7 kb genome is comprised of three ORFs. ORF1 encodes a nonstructural polypeptide consisting of p48, NTPase, p22, VPg (viral genome-linked protein), viral protease, and RNA-dependent RNA polymerase (RdRP). ORF2 encodes a major capsid protein, VP1, and ORF3 encodes a minor capsid protein, VP2. VP1 binds to the host cell receptor, histo-blood group antigens (HBGAs), promotes viral entry, and determines antigenicity and strain specificity. VP1 also elicits host protective antibody, cellular and humoral immunity; while the VP2 helps in RNA packaging, regulates the synthesis of VP1, and stabilizes the VP1 structure.

NoV belongs to the family of *Caliciviridae* that has six genera: *Norovirus*, *Sapovirus*,

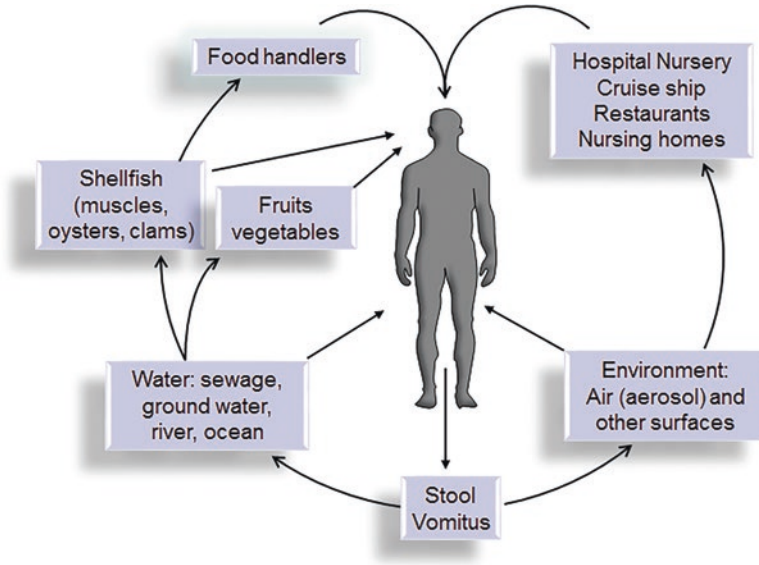

Fig. 6.4 Transmission pathway for norovirus (Schematics based on Ushijima et al. 2014. Food Safety, 2, 37–54)

Lagovirus, *Vesivirus*, *Recovirus*, and *Becovirus*. Human infections are generally associated with viruses from the genus *Norovirus*. NoV is now grouped into six genogroups (G1-GVI): GI is isolated from humans; GII from both humans and swine; GIII from cattle; GIV from human, feline, and canine; GV from murine; and GVI from the canine. Genogroups are again subdivided into genotypes. Genogroup GI has >9 while GII has >22 genotypes. GII.4 is currently the most common epidemic strain.

Pathogenesis

There is no reliable cell culture or small animal model, which can be used to study human norovirus pathogenesis or viral growth. However, gnotobiotic pigs, gnotobiotic calves, rhesus macaques, and chimpanzees have shown to respond to human norovirus infection. Feline calicivirus (FCV) and murine norovirus (MNV) have been used as surrogates. FCV is the member of family *Caliciviridae* and genus *Vesivirus*. Likewise, MNV is the member of *Caliciviridae* family and genus *Norovirus*. Both possess some attributes similar to the human norovirus, and they can be cultured in cell culture models thus, have been used widely as NoV surrogates. The infectious dose of NoV is very low: about 10–100 virus particles. Severity and the onset of disease may depend on a number of virus particles ingested. The HBGAs distributed in blood cells, vascular endothelial cells, and mucosal gastrointestinal epithelial cells serve as the receptor for viral interaction. Norovirus infects mature enterocytes covering the small intestinal villi leading to massive cell damage and malabsorption. Damaged cells are rapidly replaced by undifferentiated immature enterocytes originated from the crypt, which are not susceptible to the virus infection. These immature cells cannot function properly; thus, malabsorption continues until the cells mature. The virus causes gastroenteritis, characterized by explosive projectile vomiting, nausea, cramps, diarrhea, dehydration, anorexia, headache, chills, fever, and myalgia. Adults are more susceptible to norovirus than children are, and the incubation period is 24–48 h.

Symptoms appear within 12–24 h after ingestion and last for 2–3 days. The disease is self-limiting, and the viruses are excreted in the vomitus and feces of infected persons at the rate of 10^4–10^5 virus particles per gram of vomitus and 10^8–10^{10} particles per gram of feces. Recovering patients shed virus for an extended period even up to a month or longer. Immunocompromised patients, children, and the elderly may shed for a prolonged duration. Adults may encounter recurrent infections despite the presence of norovirus-specific antibody in the

serum. Cell-mediated immunity is Th1-dependent, and IFNγ is the dominant cytokine.

Prevention and Control

Effective sanitization and disinfection are needed to prevent and control the spread of NoV. However, it is resistant to standard sanitizers and disinfectants at the permissible levels during food processing or food production. Since it is resistant to standard chlorination treatment, so a concentration >10 mg L^{-1} is needed to disinfect water. As an industry practice for leafy green sanitization, chlorine is used at 0.2 mg L^{-1}, which is not effective against NoV; however, 15–30 s treatment with 2–5% trisodium phosphate solution (TPS) can effectively reduce viral loads on produce. Human milk, oligosaccharide, milk glycoprotein, and milk glycolipid, contain the same epitope as HBGA and can block viral binding to the epithelial cells and provide a strategic approach to preventing viral interaction with enterocytes. Currently several vaccines are under development based on viral proteins; however, due to the lack of a cell culture model to grow the virus, live attenuated viral vaccine development is not possible. Noroviruses are genetically and antigenically diverse; thus, vaccine efficacy would be limited. The virus is routinely detected by ELISA and by RT-PCR assay targeting the RNA polymerase gene. Electron microscopy is used as a confirmatory test.

Zoonotic Viral Pathogens

Avian Flu Virus

Introduction

Avian flu is also known as avian influenza and the predominant strain is H5N1. Avian influenza virus infects birds including poultry; however, it is capable of adapting and causing infection in humans, especially the poultry handlers, and thus it is considered a zoonotic pathogen (Fig. 6.5). Avian influenza virus has been isolated from wild birds such as geese, ducks, and waterfowls and mammals including pig, horse, dog, and sea mammals. The migratory birds can readily transmit the virus to different continents. Even though the avian influenza virus has been isolated from poultry meat, there is no evidence for foodborne transmission. Though human-to-human transmission has not been confirmed, scientists predict that avian influenza can possibly cause a pandemic, which is now responsible for the epidemic in Asia, Africa, and Eastern Europe. According to the World Health Organization

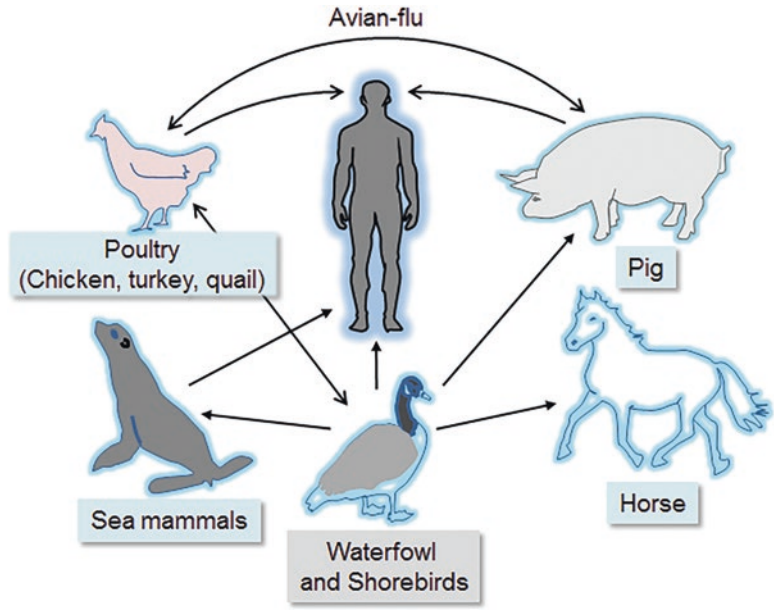

Fig. 6.5 Interspecies transmission pathways for avian flu virus (avian influenza H5N1 virus)

(WHO), between 2003 and 2015, avian influenza virus strain H5N1 infected 840 and killed 447 worldwide.

There are three types of influenza viruses: A, B, and C. Influenza virus type A is responsible for human epidemic every year, and it was responsible for past pandemics. In the human history, three major pandemics of influenza A virus have been reported: 1918 Spanish flu (H1N1), 1957 Asian flu (H2N2), and 1968 Hong Kong flu (H3N2). These three pandemics killed millions of people. The avian flu virus has an avian origin or the virus results from avian-human virus reassortment.

Biology

Avian influenza virus belongs to the family *Orthomyxoviridae* and genus *Influenzavirus A*. Influenza A virus is an enveloped virus and the size is about 80–120 nm in diameter. It is a negative-sense single-stranded RNA virus with eight different segments and encodes ten proteins including surface glycoproteins, hemagglutinin (HA), and neuraminidase (NA) and matrix proteins M2 and M1, nonstructural proteins NS1 and NS2, the nucleocapsid, and the three polymerase enzymes, PB1 (polymerase basic 1), PB2 (polymerase basic 2), and PA (polymerase acidic).

HA and NA are the surface antigens and are a determinant of the pathogenicity, transmission, and adaptation of the virus to other species. HA is the most important determinant and binds to the host epithelial cell receptor for viral entrance and replication, while NA is responsible for the release of newly formed viruses. Both HA and NA are responsible for antigenic variation resulting in antigenic drift and shift and allow the virus to evade the host immune system. The virus lacks a proofreading and correction mechanism during viral replication; hence, the error introduced due to nucleotide substitution, deletion, or point mutation results in antigenic variation. There are 16 subtypes of HA (H1–H16) and 9 subtypes of NA (N1–N9), and many of these are associated with various animals including humans, dogs, pigs, horses, and birds. The subtypes H1, H2, and H3 and N1 and N2 are associated with human infection; H1 and H3 and N1 and N2 are associated with pigs and H3 and N8 in dogs. A majority of avian influenza viruses are H5 and H7 subtypes, and H5, H7, and H9 have caused sporadic infections in humans. There are two pathotypes, highly pathogenic avian influenza (HPAI) and low pathogenic avian influenza (LPAI). Several different avian influenza virus types have been isolated from different regions or countries: H1N1 (Asia), H4N6 (Canada), H9N2 (China), and H5N1 (Asia). Avian flu virus is currently named by type/place isolated/culture number/year of isolation. For example, the strain isolated from Shanghai, China, is designated as B/Shanghai/361/2002 (H5N1).

Pathogenesis

Typically, the influenza virus affects the respiratory tract. The HA of influenza virus binds to the epithelial sialic acid-containing receptor before initiating an infection. In human, this receptor consists of sialic acid–galactose, and the cross-link between these two molecules consists of α-2,6 linkage (SA α-2,6), while in birds it is α-2,3 linkage (SA α-2,3). This difference possibly prevents the avian influenza virus from readily infecting humans. Moreover, the receptors with the α-2,3 linkages are distributed in the lower part of the respiratory tract of humans thus also reduces the access of the virus. In addition, human isolates of avian H5N1 have shown to display antigenic variation to show binding to human cellular receptor containing α-2,6 linkage. The virus infects and multiplies in nasopharyngeal and alveolar epithelial cells. The virus also exhibits tropism for the liver, renal system, and other tissues showing signs of diarrhea, renal dysfunction, and lymphopenia.

The symptoms of H5N1 infection in humans appear 2–8 days after exposure, and clinical signs include flu-like symptom, fever, cough, shortness of breath, and pneumonia affecting primarily the lower respiratory tract. The disease may progress to vomiting, diarrhea, and abdominal pain. The patient requires mechanical ventilation and dies within 9 days from the onset of symptoms.

In birds, the low infective pathotype exhibits mild symptoms characterized by ruffled feathers and mild respiratory symptoms lasting approximately for

10 days. The high infective pathotype shows severe respiratory and neurological disorders, organ failure, and death within 2–3 days.

Prevention and Control

The avian flu virus can be cultured using Madin–Darby canine kidney (MDCK) cell line or embryonated eggs (see Chap. 5). Viral antigens can be detected from a clinical specimen by enzyme immunoassay or immunofluorescence assay, and viral RNA can be detected by using a reverse transcriptase PCR (RT-PCR) assay that targets genes for HA and NA synthesis. Antiviral drugs, amantadine, rimantadine, oseltamivir and zanamivir, show serotype-specific effectiveness. NA inhibitors (oseltamivir and zanamivir) are shown to be effective against H5N1 in vitro and in a mouse model. Several vaccines based on killed subunit vaccines are under development, but those must be able to protect against different strains currently causing infections in humans globally.

Waterfowl is the natural reservoir for avian influenza, and the virus can be transferred to domestic birds by respiratory and fecal–oral routes through contaminated water, feed, environment, and feces. Infiltration of wild birds should be prevented from poultry farms or premises.

The avian flu virus has the potential for causing a pandemic; thus, precaution should be taken to prevent the spread. Contact with infected domestic or wild birds should be avoided. Routine surveillance of migratory birds (dead or alive) or birds in a poultry farm for the presence of influenza virus should be performed. Vaccination of human populations may be needed to control the spread; however, concerns of antigenic variation may challenge its efficacy and effectiveness.

Can the avian flu virus be a food safety concern? It has been demonstrated that conventional cooking temperature (70 °C or more) can readily inactivate the H5N1 virus; however, the virus may not be killed by refrigeration or freezing, and in fact, the virus has been isolated from frozen duck meat. If the poultry eggs and meats are properly cooked, they can eliminate the virus. The greatest risk of exposure is through handling and slaughter of live infected poultry.

Nipah Virus

Introduction

The first Nipah virus (NiV) outbreak was reported in Malaysia in 1998–1999, which affected 276 people and 39% fatality, and the infection was originally transmitted through exposure to infected swine. The NiV was amplified at large numbers in the respiratory tracts and facilitated the spread of infection in farm workers. NiV was originally isolated from a patient from Sungai Nipah village in Malaysia, and fruit bat (genus, *Pteropus*), also called flying fox, acts as a natural reservoir. Interaction of fruit bats with swine and humans led to increased numbers of outbreaks in Malaysia. Bats transmit the virus through saliva or urine to the fruits. Swine from a farm located near the bat habitat acquires the organism and aid in the zoonotic transmission of the disease (Fig. 6.6). Domestic animals foraging may eat virus-laden partially eaten fruits and may transmit the disease to humans. NiV is also transmitted through sap (juice) of date palm tree when fruit bats feed on sap at night, and this leads to numerous epidemic and sporadic outbreaks in Bangladesh and the eastern part of India. Date palm tree sap is used for making molasses. The virus survives well in the sap, and unheated sap can transmit the virus to humans. Person-to-person transmission also occurs. Nipah virus outbreak was also reported in Singapore, Cambodia, and Thailand, and virus has been circulating in the natural reservoir in Southeast Asia, including Malaysia, Cambodia, Indonesia, East Timor, Vietnam, Thailand, Bangladesh, India, and Papua New Guinea.

Biology

NiV is a member of the family of *Paramyxoviridae* and genus *Henipavirus*. NiV virus is about 500 nm in diameter and is larger than the typical paramyxoviruses (150–400 nm). The Nipah virus size may vary from 180 nm to 1900 nm. It is an enveloped negative-sense single-stranded RNA

virus with genome size 18.246 kb. Six structural proteins are encoded in the genome: two envelope glycoproteins F (fusion) and G (receptor binding), the nucleoprotein N, phosphoprotein P, matrix protein M, and the RNA-dependent RNA polymerase L. The G and F glycoproteins are required for viral attachment and entry into the host cells. The G protein binds to the receptor molecule, Ephrin-B2, which is expressed on neurons, smooth muscles, and endothelial cells. The F protein (546 amino acid residues) is a type I transmembrane protein and facilitates the fusion of virus and the host cell membrane during the infection. Both F and G proteins induce neutralizing antibodies. The M protein (352 amino acid residues) provides rigidity and the structural stability of the virion through its interactions with the F protein, the ribonucleoprotein (RNP) complex, and the inner surface of the virion envelope. The N protein (532 amino acid residues) helps encapsidation of the viral genome and interacts with P protein. The L protein possesses all the enzymatic activities responsible for initiation, elongation, and termination of both mRNA transcription and genome replication. P protein serves as a scaffold between the L and the encapsidated genome.

Pathogenesis

The incubation period of NiV infection is 1–2 weeks. The virus binds to the cellular receptor Ephrin B2 present on the neuron and endothelium, enters host cells, replicates, and causes cell damage. Systemic vasculitis with extensive thrombosis is seen in patients since virus attacks endothelium in the blood vessels and the CNS. The virus causes high fever, headache, myalgia, dizziness, confusion and lack of consciousness, and encephalitis. In addition, the virus causes acute respiratory tract infection, pulmonary edema, coma, and death.

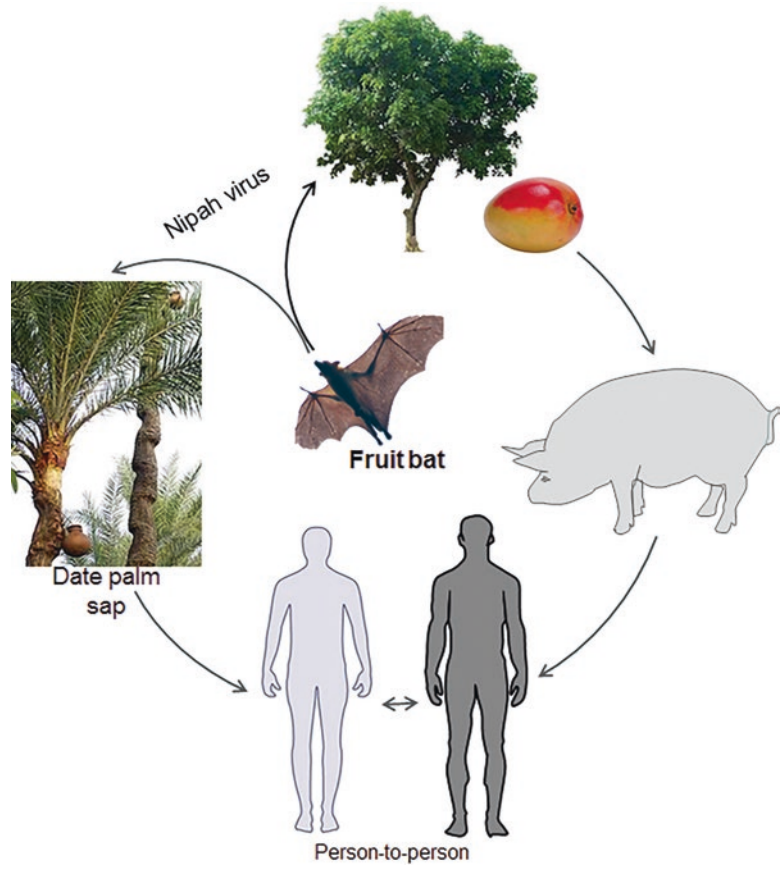

Fig. 6.6 Transmission pathways for Nipah virus

Kidneys are also affected showing signs of glomerular fibrinoid necrosis. The mortality rate in human is about 75%.

Infected bats do not show clinical signs, but serve as the carrier, whereas pigs are highly susceptible to NiV showing signs of meningitis and encephalitis, bronchointerstitial pneumonia, systemic vasculitis, and focal necrosis in the spleen and lymph nodes. Viral antigen is detected in the endothelial and smooth muscle cells of the brain, lungs, and lymphoid system. Virus antigen is present in neurons, glial cells, and epithelial cells of the upper and lower respiratory tracts.

Prevention and Control

Culling of infected swine helped reduce NiV cases in Malaysia. Heat treatment of sap or avoiding unprocessed sap consumption will also help prevent viral infection. Serologic testing for antibody titer in human sera and reverse transcriptase PCR assay have been used for diagnosis and detection from human urine, cerebrospinal fluid, and oral swabs.

Ebola Virus

Introduction

In the past several years, the Ebola virus caused major outbreaks in West and Central Africa with a case fatality rate of 25–90%. The Ebola virus was first identified in 1976 in the Democratic Republic of the Congo, and it was named after the Ebola River located near the epicenter of the first outbreak. The fruit bats are considered the reservoir. Animals or humans have acquired the disease by consuming or coming in contact with the infected bats or animals (gorillas, chimpanzees, and monkeys). Thus, there is a strong evidence for the infection to be of foodborne zoonotic disease, and bush meat may be an important link. The virus can pass through bodily fluids and spread from human-to-human.

Biology

The Ebola virus is an enveloped single-stranded RNA virus (19 kb) of the family *Filoviridae* and genus *Ebolavirus*. It is a filamentous and pleomorphic virus with about 1200 nm in length. The viral genome encodes for a nucleoprotein (NP), glycoprotein (GP), RNA-dependent RNA polymerase (L), and four structural proteins: VP24, VP30, VP35, and VP40. In addition, it expresses a truncated soluble form of GP (sGP) through RNA editing. Five strains are reported: Zaire ebolavirus (EBOV), Sudan ebolavirus (SUDV), Tai Forest ebolavirus (TAFV), Bundibugyo ebolavirus (BDBV), and Reston ebolavirus (RESTV). All are pathogenic to humans except RESTV, which is thought to be pathogenic to nonhuman primates.

Pathogenesis

The incubation period of the disease is 2–21 days (average 7–10 days). The pathogenesis is not fully understood, but the virus is thought to suppress both innate and adaptive (cellular and humoral) immune responses. The virus replicates in monocytes, macrophages, and dendritic cells. The virus is found inside endothelial cells, fibroblasts, hepatocytes, and adrenal cells and disseminates to the lymph nodes, liver, and spleen. Massive production of proinflammatory cytokines (IL-1, IL-6, IL-8, IL-15, IL-16) and several chemokines lead to shock and multi-organ failure and death. The symptoms of infection include lack of appetite, fever, headache, malaise, joint and muscle aches, abdominal pain, diarrhea and vomiting, and internal and external bleeding at the later stage of the disease. Reverse transcriptase PCR is used for diagnosis of the disease. Vaccines are being developed for humans using inactivated virus and DNA vaccine using replication-defective recombinant adenovirus type 5 expressing glycoprotein (GP) and nucleocapsid protein (NP).

Prevention and Control of Foodborne Viruses

Enteric viruses are shed in large numbers from the host through feces and vomitus, and they could be airborne or waterborne. The infectious dose is very low; thus, effective sanitization and control measures need to be employed to prevent contamination and spread. Person-to-person transmission occurs readily when people are in

close contacts, especially in cruise ships, in restaurants, and in hospitals.

Depuration helps remove the virus from shellfish; however, proper water temperature should be maintained. During depuration, harvested shellfish are kept in clean fresh water for 24–48 h where viruses are escaped into the water. Food preservatives (chlorine compounds, detergents, etc.), freeze-drying, ultraviolet light, freezing, and heating at 100 °C can inactivate foodborne viruses. In general, viruses are highly stable, because the virus coat proteins most likely provide the protection against processing treatments. Viruses are remarkably stable at high temperatures such as 90–100 °C, possibly because the virus particles may remain aggregated or protected by food particles. UV treatment can inactivate the virus. During the farming of fruits and salad vegetables, clean virus-free water should be used for irrigation. Food handlers serve as a source; thus, workers' health and hygienic practices should receive the greatest attention. Shellfish are a potential source of norovirus and hepatitis A virus, and these animals should not be harvested from water that may have been polluted with sewage.

Infective Proteins

Bovine Spongiform Encephalopathy

Introduction

A group of neurodegenerative infective agents (prions) capable of transmission to various hosts is termed transmissible spongiform encephalopathies (TSEs). Several TSE agents are described in the literature: bovine spongiform encephalopathy (BSE) or "mad cow disease" in cattle, "scrapie" in sheep and goat, chronic wasting disease (CWD) in cervids, transmissible mink encephalopathy (TME) in minks, and Creutzfeldt–Jakob disease (CJD), variant CJD (vCJD), Gerstmann–Straussler syndrome, and "kuru" in humans. In the early 1950s, in the eastern highlands of Papua New Guinea, Kuru was prevalent among the islanders due to a cannibalistic practice of consumption of infected brain tissues of relatives. The disease was characterized by the degenerative brain with spongy appearance, and the victims suffered from rapid physical and mental abnormalities, culminating in paralysis, coma, and death. It was called slow virus because the incubation period is about 2–10 years.

It became a major concern in the early 1990s when the disease was detected in cattle, and the wasting of brain tissue resulted in abnormal behavior in cattle; hence, it was called "mad cow disease." Animals exhibit symptoms of abnormal gait, hyper-responsiveness to stimuli, tremors, aggressive behavior, nervousness or apprehension, changes in temperament, and even frenzy. Cattle over 24–30 months of age are susceptible to this infection. The incubation period of classical BSE is about 2–8 years. Though there is no human case directly linking the consumption of contaminated beef, finding the organism in the late 1990s and early 2000 (2003) in Canada and the USA caused a major beef embargo among developed countries with huge economic impacts costing both countries over 4–6 billion dollars.

WHO reported that from October 1996 to March 2011, 175 cases of vCJD have been reported in the UK; 25 in France; 5 in Spain; 4 in Ireland; 3 each in the Netherlands and the USA; 2 each in Canada, Italy, and Portugal; and 1 each in Japan, Saudi Arabia, and Taiwan. BSE is endemic in the UK with reported 176 cases of vCJD as of April 2012. The incubation period for vCJD in humans is 11–12 years. Before 1980, vCJD occurred due to the use of (1) cadaveric human growth hormone, (2) contaminated surgical instruments, (3) infected dura mater graft, and (4) corneal transplant. In recent years, however, consumption of contaminated animal products with a brain, lymph nodes, or neurons is thought to be responsible for transmission. One suspected source of BSE in beef is presumably due to the feeding of beef cattle with contaminated meat and bone meal (MBM) preparation, for fast growth and increased body weight gain. MBM is often prepared from sheep offal and/or condemned bovines, which are not fit for human food. In 1997, the US-FDA banned the use of proteins derived from mammalian tissues in

feeding to ruminants in an effort to prevent transmission of TSE to food animals. Conversely, the UK delayed imposing such a ban and about 100 persons developed fatal cases of vCJD between roughly 1996 and 2005.

Biology

BSE-causing agent was originally thought to be a virus, but later in 1982, Dr. Prusiner discovered that it is a proteinaceous infectious particle called prion protein (PrP). He received the Nobel Prize for his work in 1997. Prion protein has aberrant protein folding, and its accumulation in nervous tissues leads to neurodegeneration. It is resistant to most treatments including heat, chemicals, and proteases. The prion is found primarily in the central nervous systems (CNS) including the brain and neurons and in lymphatic system in the gut. Amino acid sequences of PrP from normal and infected brains are identical but show differences in biochemical and biophysical behaviors. Monomeric form of the PrP protein contains 253 amino acids with a molecular mass of 22–36 kDa, while the abnormal or infective molecule is a macromolecular aggregate with a molecular mass greater than 400 kDa. The normal cellular version of PrP is called PrP^{c} and is encoded by a single chromosomal gene, *PRNP* located on the chromosome 20 in humans. PrP^{C} is sensitive (PrP^{sen}) to proteases such as proteinase K and trypsin. PrP-mRNA is 2.1 kb long and is detected primarily in the brain (neurons) and small amounts in the lungs, spleen, and heart. The infective form is resistant to protease and has a drastically different secondary structure and referred to as PrP^{Sc} (PrP from scrapie). The α helical structures are predominant in PrP^{C}, while a misfolding of the prion protein results in the formation of β-sheet, which is abundant in PrP^{Sc}. Normal PrP^{c} has α-helix of 40% and β-sheet 3% while in the disease-causing prion (PrP^{Sc}) has α-helix 30% and β-sheet 40%. The prions are highly hydrophobic and form aggregates easily. Aggregates are highly resistant to cellular digestion and accumulate in the lymphoid and nerve tissues and cause a spongiform change in the brain.

Prion (PrP^{Sc}) is highly resistant to heat, certain chemicals, and proteases (proteinase K, trypsin, etc). It can withstand dry heat treatments of 160 °C for 24 h, or at 360 °C for 1 h, and saturated steam autoclaving at 121 °C for 1 h. The prion protein is resistant to chemical treatments such as 0.5% sodium hypochlorite for 1 h, 3% hydrogen peroxide for 1 h, and ethanol. However, complete inactivation is possible by autoclaving at 132 °C for 1.5 h, and treatment with 1 M sodium hydroxide at 20 °C for 1 h, or sodium hypochlorite (2% chloride) for 1 h at 20 °C.

Pathogenesis

Transmission of prion through digestive tract has been the subject of much investigation in recent years. Prion possibly passes through the M cells overlying the Peyer's patches, and it is then transported by the dendritic cells to the central nervous system and the brain. In another study, it is proposed that the prion bypasses the lymphoid system altogether and is directly transmitted via the peripheral nervous system to reach the CNS. PrP accumulates in the neural cells and disrupts normal neurological function, causing vacuolation (spongy appearance) and cell death.

In humans, the first signs are psychiatric, such as anxiety, depression, insomnia, withdrawal, paranoid delusions, head and neck pain, and progressive dementia. Mean duration of suffering is about 14 months. The neurologic symptom is accompanied by cerebral ataxia (defective muscular coordination) and dementia. In the terminal stage, the patient becomes bedbound, akinetic, and mute, a state in which the person is not able or will not move or make sounds.

Prevention and Control

There is no laboratory test available to use in the live animals or humans for testing of abnormal PrP^{Sc}. Postmortem analysis of brain tissues shows characteristics amyloid plaque, which is a waxy translucent substance, composed of complex protein fibers and polysaccharides that are formed in body tissues in some degenerative diseases, such as Alzheimer's disease, and spongy appearance. Immunoassays (Western blot or ELISA) are used to detect PrP^{Sc} antigens in cattle after slaughter. In humans, magnetic resonance imaging (MRI) has been used as a tentative diagnosis to detect cortical atrophy in the brain, coupled with

clinical signs. There is no treatment available for the infectious prion.

PrP^{Sc} is concentrated in certain tissues in infected animals and referred to as SRM (specific risk material). These include the brain, spinal cord, skull, vertebral column, eyes, tonsil, and ileum. BSE can be prevented by several ways: (1) routine surveillance for BSE-infected cattle, (2) prevent entry of BSE agent in cattle population, (3) stop feeding the beef cattle with animal proteins derived from other animals, and (4) identify and condemn the infected cattle before entering into the human food chain. Currently, the European Union and the USA have banned the feeding of animal proteins from other animals. BSE suspect carcasses should not be used for food, and carcasses should be destroyed at 133 °C (under pressure) for 20 min.

Summary

Foodborne viruses such as rotavirus, norovirus, and hepatitis A virus cause enteric disease characterized by gastroenteritis and other complications and affect a large number of people every year. Foodborne enteric viruses are generally RNA virus, and they are shed in large numbers (about 10^9 particles per gram) from infected patients through vomitus and feces. Person-to-person or fecal–oral transmission is a common mechanism for viral infection. Since viruses are highly infectious, only a small dose of 10–100 particles is sufficient to cause an infection. Zoonotic viral pathogens are transmitted from animals and avian species to humans, sometimes through direct contact with the animal or the meat. Avian influenza virus is transmitted primarily through contact or aerosol to the bird handlers and is not considered a foodborne pathogen, but it has the potential to cause human pandemics. Avian flu virus infection is fatal and affects lower respiratory tract resulting in pneumonia. The Nipah virus is transmitted by fruit bat while feeding on fruits or palm sap, and it is responsible for viral transmission to swine or directly to humans. It has a very high mortality rate. Likewise, Ebola virus is a zoonotic viral pathogen with 25–90% mortality rate. The infection may be the foodborne source, but the virus can spread from human-to-human through bodily fluids. Transmissible spongiform encephalopathy (TSE) diseases such as bovine spongiform encephalopathy (BSE) and varient Creutzfeldt-Jakob Disease (vCJD) are caused by misfolded neurodegenerative infectious prion proteins (PrP^{Sc}), which are highly resistant to heat and protease enzymes and can be transmitted by consuming contaminated meat. Preventing the use of meat–bone meal (MBM) or specific risk materials (SRM) can prevent the spread of prions among the meat-producing animals and to humans.

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