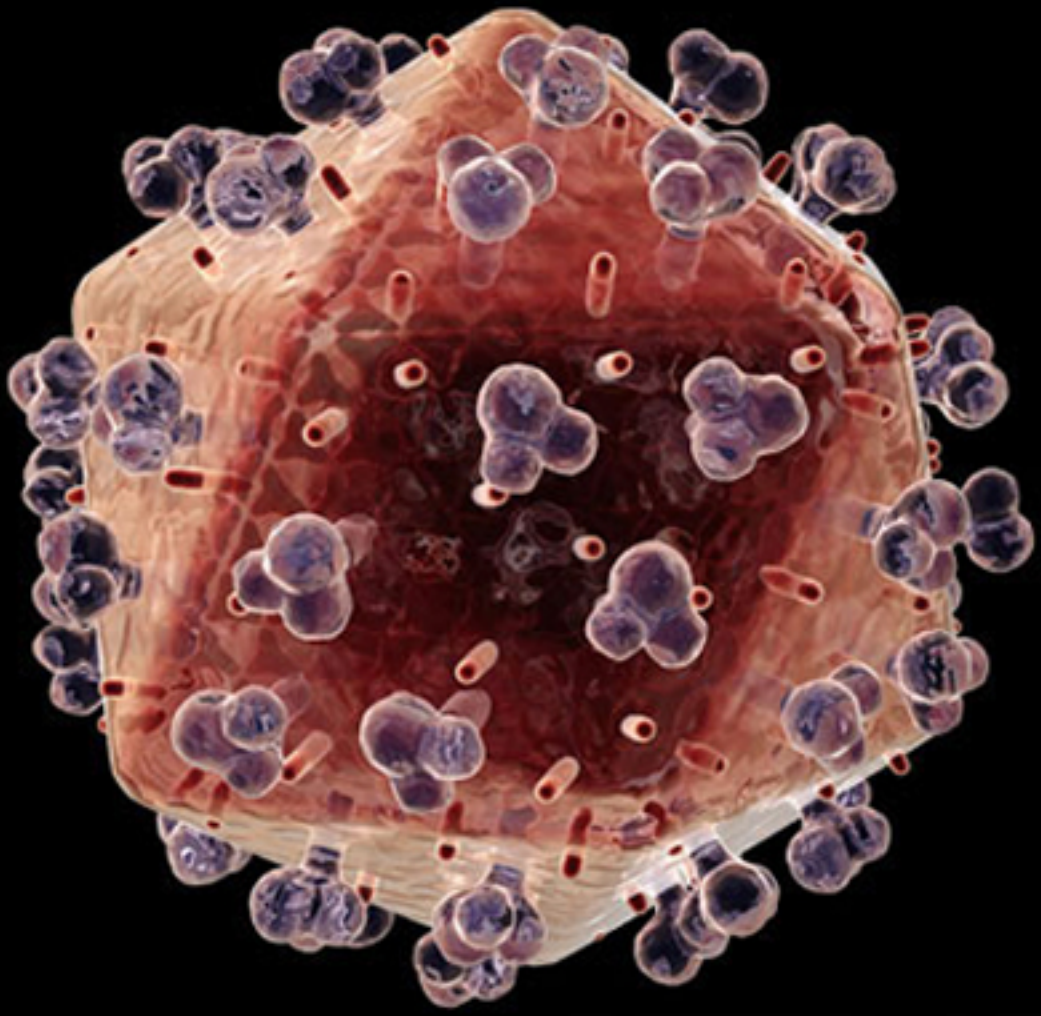
Common Viral Infections and Foodborne Illnesses



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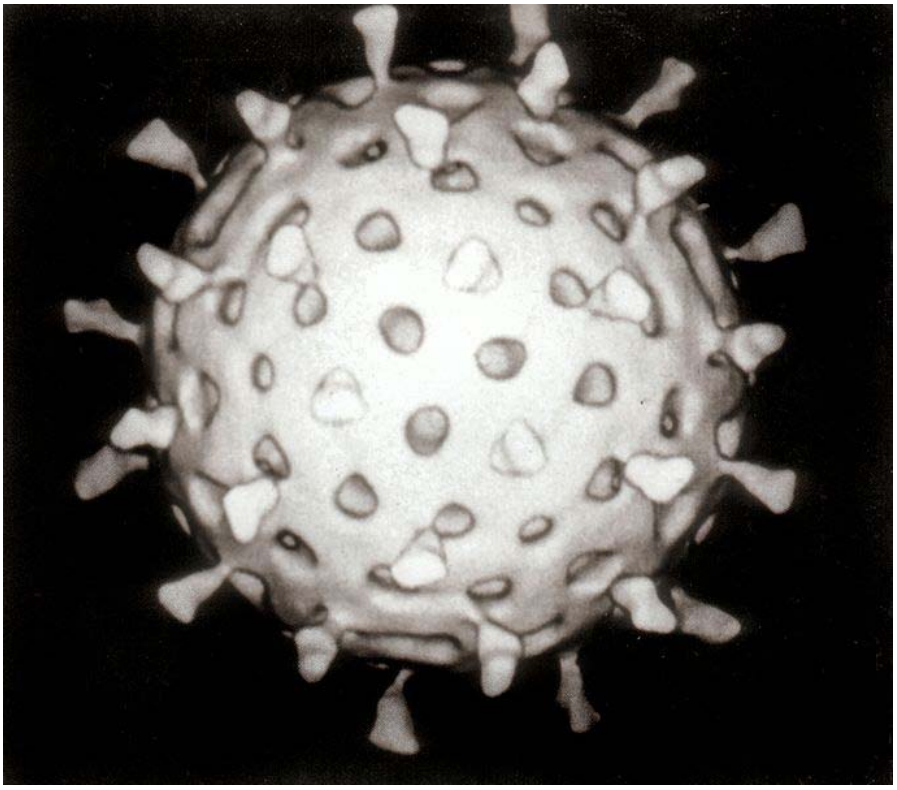
Virus

Viruses



Rotavirus

Virus classification

Group: I–VII

Groups

I: dsDNA viruses
II: ssDNA viruses
III: dsRNA viruses
IV: (+)ssRNA viruses
V: (−)ssRNA viruses
VI: ssRNA-RT viruses
VII: dsDNA-RT viruses

A **virus** is a small infectious agent that can replicate only inside the living cells of organisms. Most viruses are too small to be seen directly with a light microscope. Viruses infect all types of organisms, from animals and plants to bacteria and archaea. Since the initial discovery of the tobacco mosaic virus by Martinus Beijerinck in 1898, about 5,000 viruses have been described in detail, although there are millions of different types. Viruses are found in almost every ecosystem on Earth and are the most abundant type of biological entity. The study of viruses is known as virology, a sub-speciality of microbiology.

Virus particles (known as *virions*) consist of two or three parts: the genetic material made from either DNA or RNA, long molecules that carry genetic information; a protein coat that protects these genes; and in some cases an envelope of lipids that surrounds the protein coat when they are outside a cell. The shapes of viruses range from simple helical and icosahedral forms to more complex structures. The average virus is about one one-hundredth the size of the average bacterium.

The origins of viruses in the evolutionary history of life are unclear: some may have evolved from plasmids – pieces of DNA that can move between cells – while others may have evolved from bacteria. In evolution, viruses are an important means of horizontal gene transfer, which increases genetic diversity.

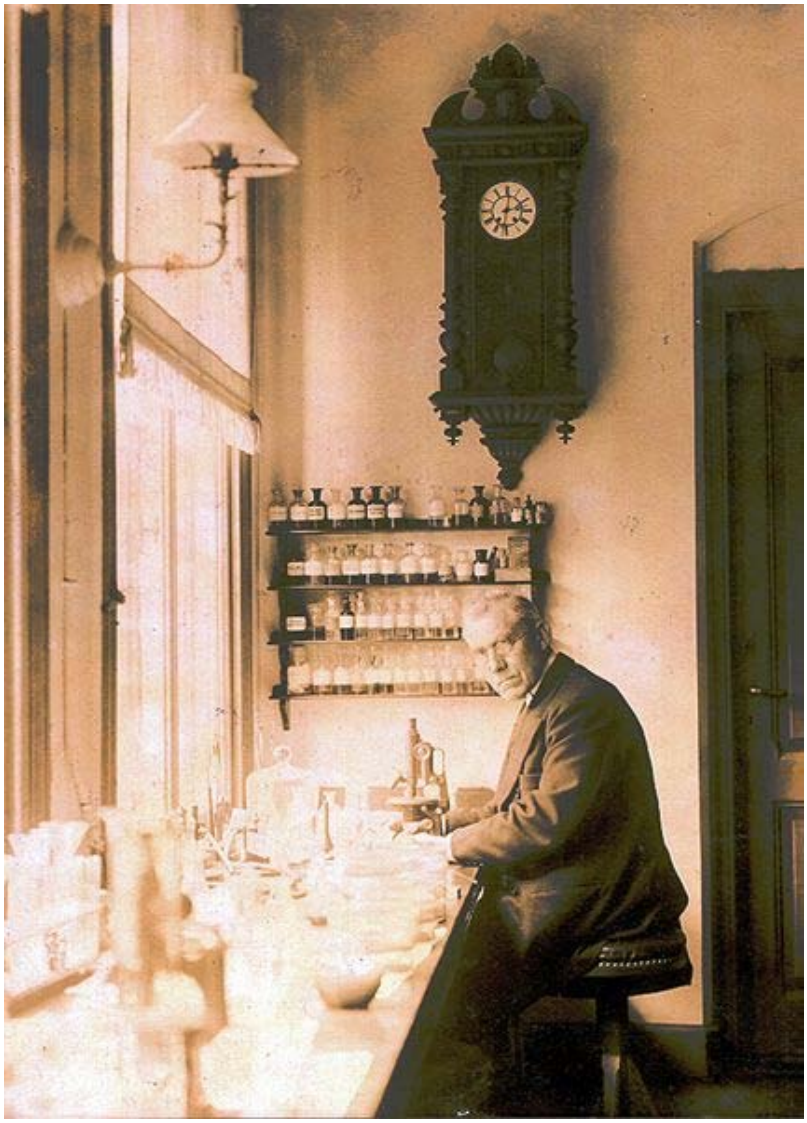
Viruses spread in many ways; plant viruses are often transmitted from plant to plant by insects that feed on sap, such as aphids, while animal viruses can be carried by blood-sucking insects. These disease-bearing organisms are known as vectors. Influenza viruses are spread by coughing and sneezing. The norovirus and rotavirus, common causes of viral gastroenteritis, are transmitted by the faecal-oral route and are passed from person to person by contact, entering the body in food or water. HIV is one of several viruses transmitted through sexual contact and by exposure to infected blood. Viruses can infect only a limited range of host cells called the "host range". This can be narrow or, as when a virus is capable of infecting many species, broad.

Viral infections in animals provoke an immune response that usually eliminates the infecting virus. Immune responses can also be produced by vaccines, which confer an artificially acquired immunity to the specific viral infection. However, some viruses including those causing AIDS and viral hepatitis evade these immune responses and result in chronic infections. Antibiotics have no effect on viruses, but several antiviral drugs have been developed.

Etymology

The word is from the Latin *virus* referring to poison and other noxious substances, first used in English in 1392. *Virulent*, from Latin *virulentus* (poisonous), dates to 1400. A meaning of "agent that causes infectious disease" is first recorded in 1728, before the discovery of viruses by Dmitry Ivanovsky in 1892. The plural is *viruses*. The adjective *viral* dates to 1948. The term *virion* is also used to refer to a single infective viral particle.

History



Martinus Beijerinck in his laboratory in 1921

Louis Pasteur was unable to find a causative agent for rabies and speculated about a pathogen too small to be detected using a microscope. In 1884, the French microbiologist Charles Chamberland invented a filter (known today as the Chamberland filter or Chamberland-Pasteur filter) with pores smaller than bacteria. Thus, he could pass a solution containing bacteria through the filter and completely remove them from the solution. In 1892, the Russian biologist Dmitry Ivanovsky used this filter to study what is now known as the tobacco mosaic virus. His experiments showed that crushed leaf extracts from infected tobacco plants remain infectious after filtration. Ivanovsky suggested the infection might be caused by a toxin produced by bacteria, but did not pursue the idea. At the time it was thought that all infectious agents could be retained by filters and grown on a nutrient medium – this was part of the germ theory of disease. In

1898, the Dutch microbiologist Martinus Beijerinck repeated the experiments and became convinced that the filtered solution contained a new form of infectious agent. He observed that the agent multiplied only in cells that were dividing, but as his experiments did not show that it was made of particles, he called it a *contagium vivum fluidum* (soluble living germ) and re-introduced the word *virus*. Beijerinck maintained that viruses were liquid in nature, a theory later discredited by Wendell Stanley, who proved they were particulate. In the same year Friedrich Loeffler and Frosch passed the first animal virus – agent of foot-and-mouth disease (aphthovirus) – through a similar filter.

In the early 20th century, the English bacteriologist Frederick Twort discovered a group of viruses that infect bacteria, now called bacteriophages (or commonly *phages*), and the French-Canadian microbiologist Félix d'Herelle described viruses that, when added to bacteria on agar, would produce areas of dead bacteria. He accurately diluted a suspension of these viruses and discovered that the highest dilutions (lowest virus concentrations), rather than killing all the bacteria, formed discrete areas of dead organisms. Counting these areas and multiplying by the dilution factor allowed him to calculate the number of viruses in the original suspension. Phages were heralded as a potential treatment for diseases such as typhoid and cholera, but their promise was forgotten with the development of penicillin. The study of phages provided insights into the switching on and off of genes, and a useful mechanism for introducing foreign genes into bacteria.

By the end of the 19th century, viruses were defined in terms of their infectivity, their ability to be filtered, and their requirement for living hosts. Viruses had been grown only in plants and animals. In 1906, Ross Granville Harrison invented a method for growing tissue in lymph, and, in 1913, E. Steinhardt, C. Israeli, and R. A. Lambert used this method to grow vaccinia virus in fragments of guinea pig corneal tissue. In 1928, H. B. Maitland and M. C. Maitland grew vaccinia virus in suspensions of minced hens' kidneys. Their method was not widely adopted until the 1950s, when poliovirus was grown on a large scale for vaccine production.

Another breakthrough came in 1931, when the American pathologist Ernest William Goodpasture grew influenza and several other viruses in fertilized chickens' eggs. In 1949, John F. Enders, Thomas Weller, and Frederick Robbins grew polio virus in cultured human embryo cells, the first virus to be grown without using solid animal tissue or eggs. This work enabled Jonas Salk to make an effective polio vaccine.

The first images of viruses were obtained upon the invention of electron microscopy in 1931 by the German engineers Ernst Ruska and Max Knoll. In 1935, American biochemist and virologist Wendell Meredith Stanley examined the tobacco mosaic virus and found it was mostly made of protein. A short time later, this virus was separated into protein and RNA parts. The tobacco mosaic virus was the first to be crystallised and its structure could therefore be elucidated in detail. The first X-ray diffraction pictures of the crystallised virus were obtained by Bernal and Fankuchen in 1941. On the basis of her pictures, Rosalind Franklin discovered the full DNA structure of the virus in 1955. In the same year, Heinz Fraenkel-Conrat and Robley Williams showed that purified tobacco

mosaic virus RNA and its coat protein can assemble by themselves to form functional viruses, suggesting that this simple mechanism was probably the means through which viruses were created within their host cells.

The second half of the 20th century was the golden age of virus discovery and most of the 2,000 recognised species of animal, plant, and bacterial viruses were discovered during these years. In 1957, equine arterivirus and the cause of Bovine virus diarrhea (a pestivirus) were discovered. In 1963, the hepatitis B virus was discovered by Baruch Blumberg, and in 1965, Howard Temin described the first retrovirus. Reverse transcriptase, the key enzyme that retroviruses use to translate their RNA into DNA, was first described in 1970, independently by Howard Martin Temin and David Baltimore. In 1983 Luc Montagnier's team at the Pasteur Institute in France, first isolated the retrovirus now called HIV.

Origins

Viruses are found wherever there is life and have probably existed since living cells first evolved. The origin of viruses is unclear because they do not form fossils, so molecular techniques have been used to compare the DNA or RNA of viruses and are a useful means of investigating how they arose. There are three main hypotheses that try to explain the origins of viruses:

Regressive hypothesis
: Viruses may have once been small cells that parasitised larger cells. Over time, genes not required by their parasitism were lost. The bacteria rickettsia and chlamydia are living cells that, like viruses, can reproduce only inside host cells. They lend support to this hypothesis, as their dependence on parasitism is likely to have caused the loss of genes that enabled them to survive outside a cell. This is also called the *degeneracy hypothesis*, or *reduction hypothesis*.

Cellular origin hypothesis
: Some viruses may have evolved from bits of DNA or RNA that "escaped" from the genes of a larger organism. The escaped DNA could have come from plasmids (pieces of naked DNA that can move *between* cells) or transposons (molecules of DNA that replicate and move around to different positions *within* the genes of the cell). Once called "jumping genes", transposons are examples of mobile genetic elements and could be the origin of some viruses. They were discovered in maize by Barbara McClintock in 1950. This is sometimes called the *vagrancy hypothesis*, or the *escape hypothesis*.

Coevolution hypothesis
: This is also called the *virus-first hypothesis* and proposes that viruses may have evolved from complex molecules of protein and nucleic acid at the same time as cells first appeared on earth and would have been dependent on cellular life for billions of years. Viroids are molecules of RNA that are not classified as viruses because they lack a protein coat. However, they have characteristics that are common to several viruses and are often called subviral agents. Viroids are important pathogens of plants. They do not code for proteins but interact with the

host cell and use the host machinery for their replication. The hepatitis delta virus of humans has an RNA genome similar to viroids but has a protein coat derived from hepatitis B virus and cannot produce one of its own. It is therefore a defective virus and cannot replicate without the help of hepatitis B virus. Similarly, the virophage 'sputnik' is dependent on mimivirus, which infects the protozoan *Acanthamoeba castellanii*. These viruses that are dependent on the presence of other virus species in the host cell are called *satellites* and may represent evolutionary intermediates of viroids and viruses.

Historically, there were problems with all of these hypotheses: The regressive hypothesis did not explain why even the smallest of cellular parasites do not resemble viruses in any way. The escape hypothesis did not explain the complex capsids and other structures on virus particles. And, the virus-first hypothesis contravened the definition of viruses in that they require host cells. However, viruses are now recognised as ancient and to have origins that pre-date the divergence of life into the three domains. This discovery has led modern virologists to reconsider and re-evaluate these three classical hypotheses.

The evidence for an ancestral world of RNA cells and computer analysis of viral and host DNA sequences are giving a better understanding of the evolutionary relationships between different viruses and may help identify the ancestors of modern viruses. To date, such analyses have not proved which of these hypotheses are correct. However, it seems unlikely that all currently known viruses have a common ancestor and viruses have probably arisen numerous times in the past by one or more mechanisms.

Prions are infectious protein molecules that do not contain DNA or RNA. They cause an infection in sheep called scrapie and cattle bovine spongiform encephalopathy ("mad cow" disease). In humans they cause kuru and Creutzfeldt-Jakob disease. They are able to replicate because some proteins can exist in two different shapes and the prion changes the normal shape of a host protein into the prion shape. This starts a chain reaction where each prion protein converts many host proteins into more prions, and these new prions then go on to convert even more protein into prions. Although they are fundamentally different from viruses and viroids, their discovery gives credence to the idea that viruses could have evolved from self-replicating molecules.

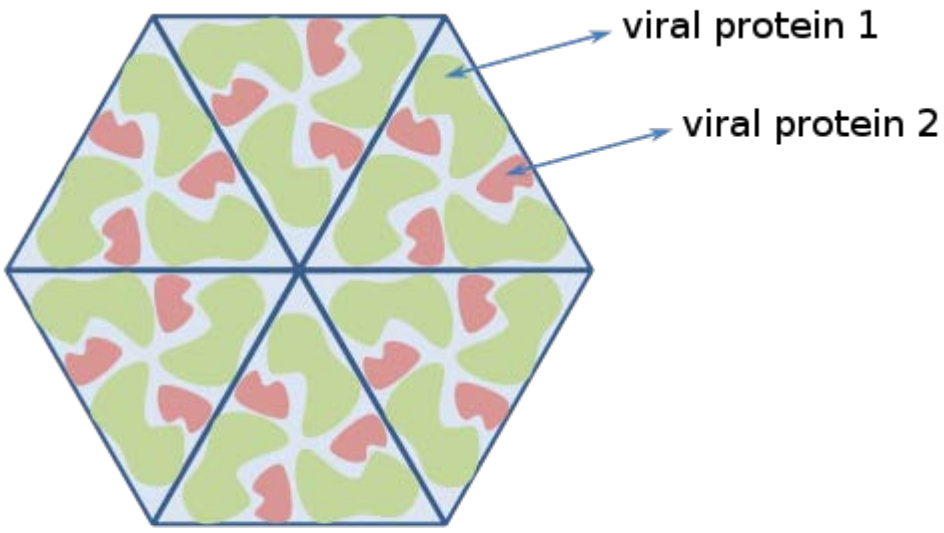
Microbiology

Life properties

Opinions differ on whether viruses are a form of life, or organic structures that interact with living organisms. They have been described as "organisms at the edge of life", since they resemble organisms in that they possess genes and evolve by natural selection, and reproduce by creating multiple copies of themselves through self-assembly. Although they have genes, they do not have a cellular structure, which is often seen as the basic unit of life. Viruses do not have their own metabolism, and require a host cell to make new products. They therefore cannot naturally reproduce outside a host cell – although bacterial species such as rickettsia and chlamydia are considered living organisms despite

the same limitation. Accepted forms of life use cell division to reproduce, whereas viruses spontaneously assemble within cells. They differ from autonomous growth of crystals as they inherit genetic mutations while being subject to natural selection. Virus self-assembly within host cells has implications for the study of the origin of life, as it lends further credence to the hypothesis that life could have started as self-assembling organic molecules.

Structure

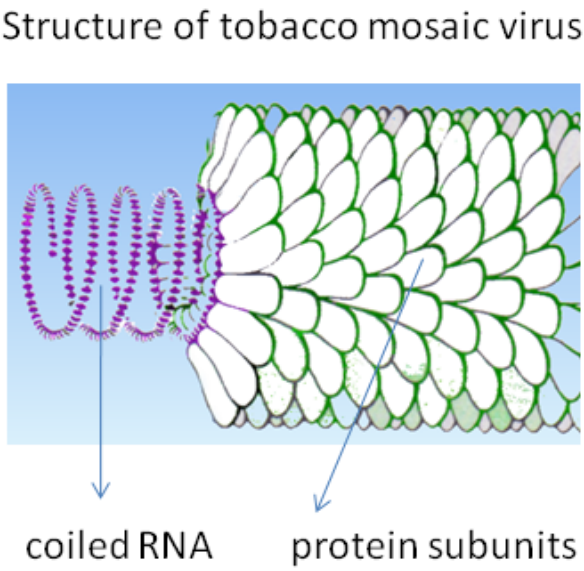


Hexon of a virus capsid made from two protein molecules

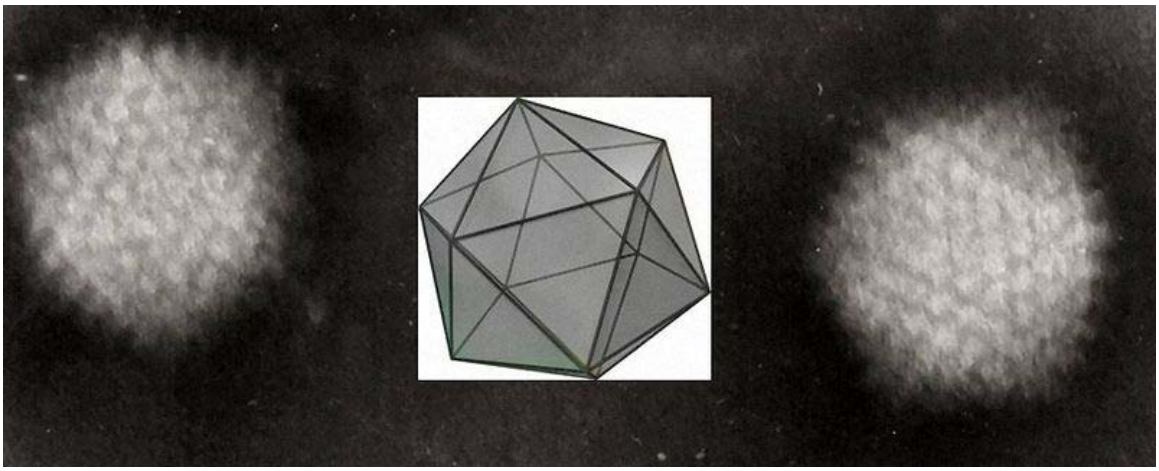
Diagram of how a virus capsid can be constructed using multiple copies of just two protein molecules

Viruses display a wide diversity of shapes and sizes, called *morphologies*. Generally viruses are much smaller than bacteria. Most viruses that have been studied have a diameter between 10 and 300 nanometres. Some filoviruses have a total length of up to 1400 nm; their diameters are only about 80 nm. Most viruses cannot be seen with a light microscope so scanning and transmission electron microscopes are used to visualise virions. To increase the contrast between viruses and the background, electron-dense "stains" are used. These are solutions of salts of heavy metals, such as tungsten, that scatter the electrons from regions covered with the stain. When virions are coated with stain (positive staining), fine detail is obscured. Negative staining overcomes this problem by staining the background only.

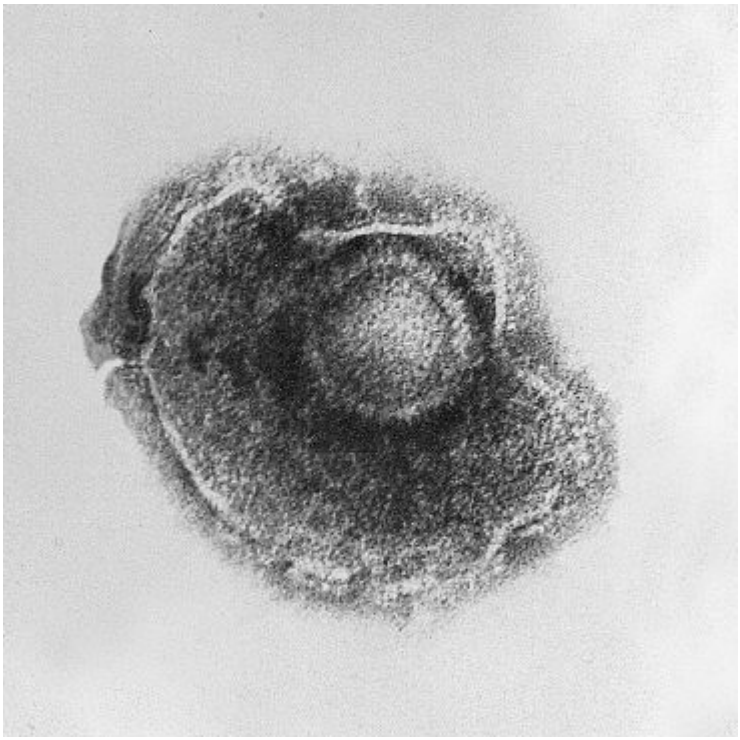
A complete virus particle, known as a virion, consists of nucleic acid surrounded by a protective coat of protein called a capsid. These are formed from identical protein subunits called capsomers. Viruses can have a lipid "envelope" derived from the host cell membrane. The capsid is made from proteins encoded by the viral genome and its shape serves as the basis for morphological distinction. Virally coded protein subunits will self-assemble to form a capsid, generally requiring the presence of the virus genome. Complex viruses code for proteins that assist in the construction of their capsid. Proteins associated with nucleic acid are known as nucleoproteins, and the association of viral capsid proteins with viral nucleic acid is called a nucleocapsid. The capsid and entire virus structure can be mechanically (physically) probed through atomic force microscopy. In general, there are four main morphological virus types:



RNA coiled in a helix of repeating protein sub-units



Electron micrograph of icosahedral adenovirus



Herpes viruses have a lipid envelope

Helical

These viruses are composed of a single type of capsomer stacked around a central axis to form a helical structure, which may have a central cavity, or hollow tube. This arrangement results in rod-shaped or filamentous virions: these can be short and highly rigid, or long and very flexible. The genetic material, generally single-stranded RNA, but ssDNA in some cases, is bound into the protein helix by interactions between the negatively charged nucleic acid and positive charges on the protein. Overall, the length of a helical capsid is related to the length of the nucleic acid contained within it and the diameter is dependent on the size and arrangement of capsomers. The well-studied tobacco mosaic virus is an example of a helical virus.

Icosahedral

Most animal viruses are icosahedral or near-spherical with icosahedral symmetry. A regular icosahedron is the optimum way of forming a closed shell from identical sub-units. The minimum number of identical capsomers required is twelve, each composed of five identical sub-units. Many viruses, such as rotavirus, have more than twelve capsomers and appear spherical but they retain this symmetry. Capsomers at the apices are surrounded by five other capsomers and are called pentons. Capsomers on the triangular faces are surrounded by six others and are call hexons.

Envelope

Some species of virus envelop themselves in a modified form of one of the cell membranes, either the outer membrane surrounding an infected host cell, or internal membranes such as nuclear membrane or endoplasmic reticulum, thus

gaining an outer lipid bilayer known as a viral envelope. This membrane is studded with proteins coded for by the viral genome and host genome; the lipid membrane itself and any carbohydrates present originate entirely from the host. The influenza virus and HIV use this strategy. Most enveloped viruses are dependent on the envelope for their infectivity.

Complex

These viruses possess a capsid that is neither purely helical, nor purely icosahedral, and that may possess extra structures such as protein tails or a complex outer wall. Some bacteriophages, such as Enterobacteria phage T4 have a complex structure consisting of an icosahedral head bound to a helical tail, which may have a hexagonal base plate with protruding protein tail fibres. This tail structure acts like a molecular syringe, attaching to the bacterial host and then injecting the viral genome into the cell.

The poxviruses are large, complex viruses that have an unusual morphology. The viral genome is associated with proteins within a central disk structure known as a nucleoid. The nucleoid is surrounded by a membrane and two lateral bodies of unknown function. The virus has an outer envelope with a thick layer of protein studded over its surface. The whole virion is slightly pleiomorphic, ranging from ovoid to brick shape. Mimivirus is the largest known virus, with a capsid diameter of 400 nm. Protein filaments measuring 100 nm project from the surface. The capsid appears hexagonal under an electron microscope, therefore the capsid is probably icosahedral.

Some viruses that infect Archaea have complex structures that are unrelated to any other form of virus, with a wide variety of unusual shapes, ranging from spindle-shaped structures, to viruses that resemble hooked rods, teardrops or even bottles. Other archaeal viruses resemble the tailed bacteriophages, and can have multiple tail structures.

Genome

Genomic diversity among viruses

Property	Parameters
Nucleic acid	• DNA • RNA • Both DNA and RNA (at different stages in the life cycle)
Shape	• Linear • Circular • Segmented
Strandedness	• Single-

	stranded • Double-stranded • Double-stranded with regions of single-strandedness
Sense	• Positive sense (+) • Negative sense (−) • Ambisense (+/−)

An enormous variety of genomic structures can be seen among viral species; as a group they contain more structural genomic diversity than plants, animals, archaea, or bacteria. There are millions of different types of viruses, although only about 5,000 of them have been described in detail. A virus has either DNA or RNA genes and is called a DNA virus or a RNA virus respectively. The vast majority of viruses have RNA genomes. Plant viruses tend to have single-stranded RNA genomes and bacteriophages tend to have double-stranded DNA genomes.

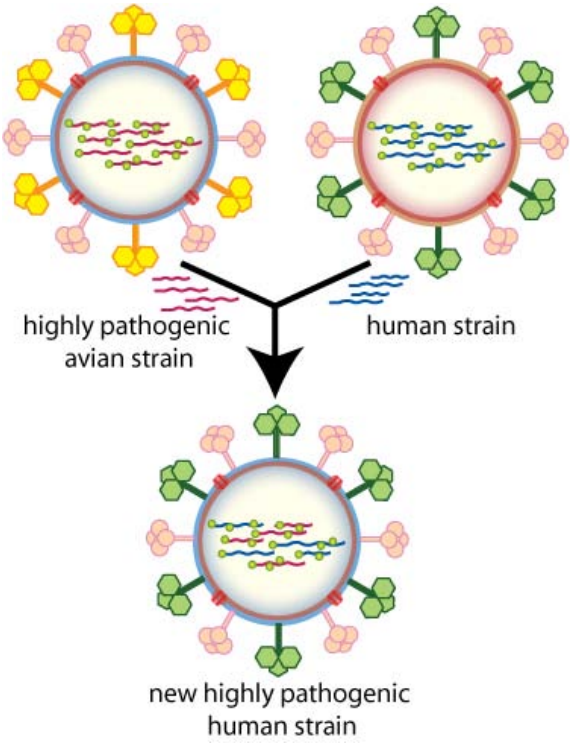
Viral genomes are circular, as in the polyomaviruses, or linear, as in the adenoviruses. The type of nucleic acid is irrelevant to the shape of the genome. Among RNA viruses and certain DNA viruses, the genome is often divided up into separate parts, in which case it is called *segmented*. For RNA viruses, each segment often codes for only one protein and they are usually found together in one capsid. However, all segments are not required to be in the same virion for the virus to be infectious, as demonstrated by brome mosaic virus and several other plant viruses.

A viral genome, irrespective of nucleic acid type, is almost always either single-stranded or double-stranded. Single-stranded genomes consist of an unpaired nucleic acid, analogous to one-half of a ladder split down the middle. Double-stranded genomes consist of two complementary paired nucleic acids, analogous to a ladder. The virus particles of some virus families, such as those belonging to the *Hepadnaviridae*, contain a genome that is partially double-stranded and partially single-stranded.

For most viruses with RNA genomes and some with single-stranded DNA genomes, the single strands are said to be either positive-sense (called the plus-strand) or negative-sense (called the minus-strand), depending on whether or not they are complementary to the viral messenger RNA (mRNA). Positive-sense viral RNA is in the same sense as viral mRNA and thus at least a part of it can be immediately translated by the host cell. Negative-sense viral RNA is complementary to mRNA and thus must be converted to

positive-sense RNA by an RNA-dependent RNA polymerase before translation. DNA nomenclature for viruses with single-sense genomic ssDNA is similar to RNA nomenclature, in that the *coding strand* for the viral mRNA is complementary to it (−), and the *non-coding strand* is a copy of it (+). However, several types of ssDNA and ssRNA viruses have genomes which are ambisense in that transcription can occur off both strands in a double-stranded replicative intermediate. Examples include geminiviruses, which are ssDNA plant viruses and arenaviruses, which are ssRNA viruses of animals.

Genome size varies greatly between species. The smallest viral genomes – the ssDNA circoviruses, family *Circoviridae* – code for only two proteins and have a genome size of only 2 kilobases; the largest – mimiviruses – have genome sizes of over 1.2 megabases and code for over one thousand proteins. RNA viruses generally have smaller genome sizes than DNA viruses because of a higher error-rate when replicating, and have a maximum upper size limit. Beyond this limit, errors in the genome when replicating render the virus useless or uncompetitive. To compensate for this, RNA viruses often have segmented genomes – the genome is split into smaller molecules – thus reducing the chance that an error in a single-component genome will incapacitate the entire genome. In contrast, DNA viruses generally have larger genomes because of the high fidelity of their replication enzymes. Single-strand DNA viruses are an exception to this rule, however, as mutation rates for these genomes can approach the extreme of the ssRNA virus case.



How antigenic shift, or reassortment, can result in novel and highly pathogenic strains of human influenza

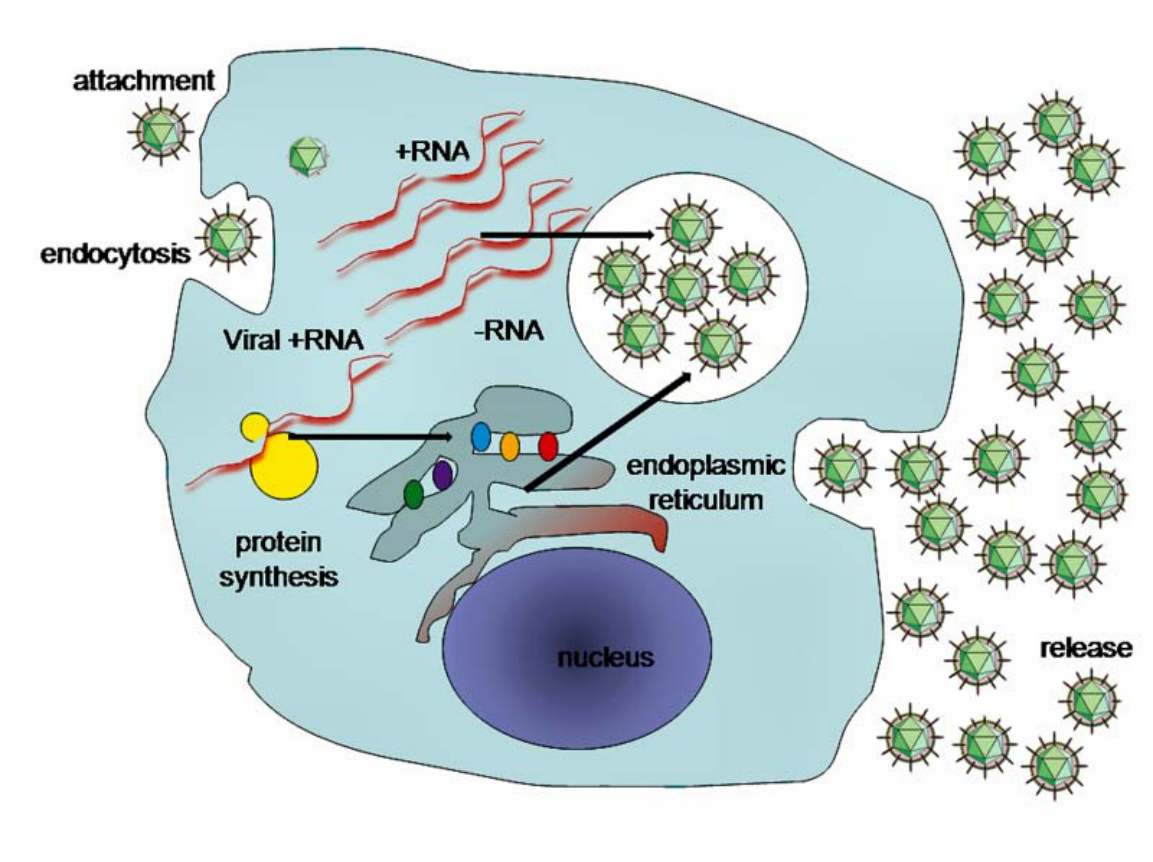
Viruses undergo genetic change by several mechanisms. These include a process called genetic drift where individual bases in the DNA or RNA mutate to other bases. Most of these point mutations are "silent" – they do not change the protein that the gene encodes – but others can confer evolutionary advantages such as resistance to antiviral drugs. Antigenic shift occurs when there is a major change in the genome of the virus. This can be a result of recombination or reassortment. When this happens with influenza viruses, pandemics might result. RNA viruses often exist as quasispecies or swarms of viruses of the same species but with slightly different genome nucleoside sequences. Such quasispecies are a prime target for natural selection.

Segmented genomes confer evolutionary advantages; different strains of a virus with a segmented genome can shuffle and combine genes and produce progeny viruses or (offspring) that have unique characteristics. This is called reassortment or *viral sex*.

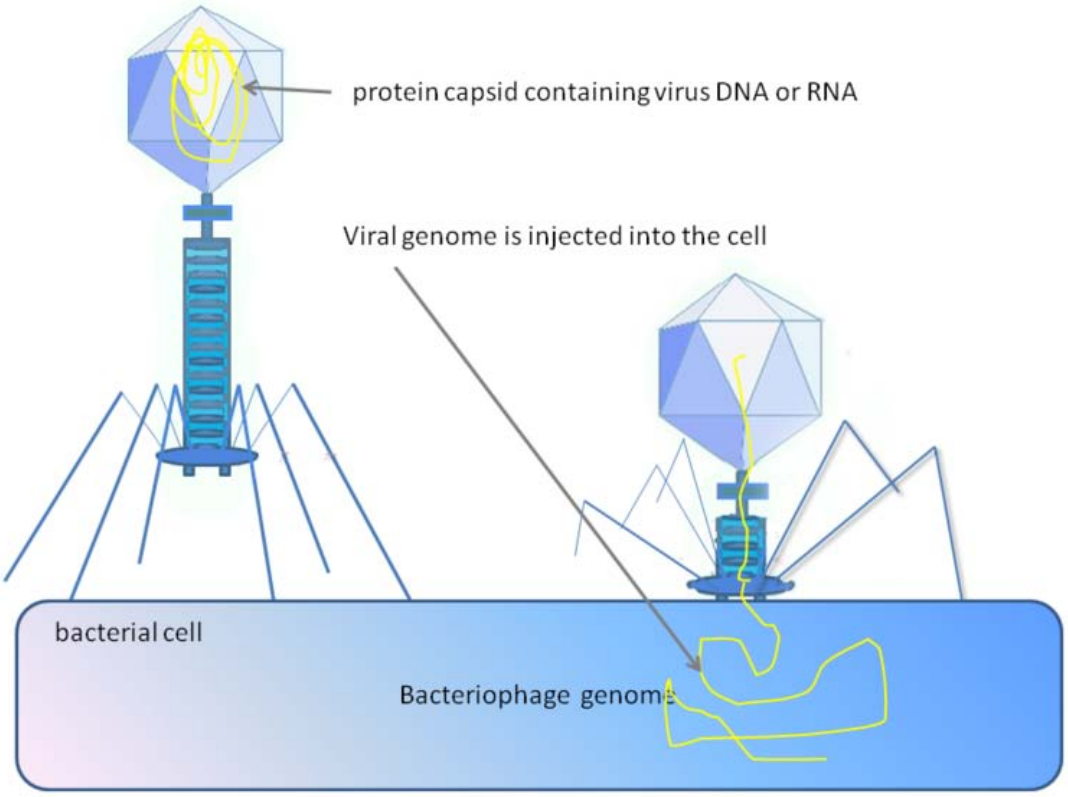
Genetic recombination is the process by which a strand of DNA is broken and then joined to the end of a different DNA molecule. This can occur when viruses infect cells simultaneously and studies of viral evolution have shown that recombination has been rampant in the species studied. Recombination is common to both RNA and DNA viruses.

Replication cycle

Viral populations do not grow through cell division, because they are acellular. Instead, they use the machinery and metabolism of a host cell to produce multiple copies of themselves, and they *assemble* in the cell.



A typical virus replication cycle



Some bacteriophages inject their genomes into bacterial cells

The life cycle of viruses differs greatly between species but there are six *basic* stages in the life cycle of viruses:

- *Attachment* is a specific binding between viral capsid proteins and specific receptors on the host cellular surface. This specificity determines the host range of a virus. For example, HIV infects a limited range of human leucocytes. This is because its surface protein, gp120, specifically interacts with the CD4 molecule – a chemokine receptor – which is most commonly found on the surface of CD4+ T-Cells. This mechanism has evolved to favour those viruses that only infect cells in which they are capable of replication. Attachment to the receptor can induce the viral envelope protein to undergo changes that results in the fusion of viral and cellular membranes, or changes of non-enveloped virus surface proteins that allow the virus to enter.
- *Penetration* follows attachment: virions enter the host cell through receptor mediated endocytosis or membrane fusion. This is often called viral entry. The infection of plant and probably fungal cells is different from that of animal cells. Plants have a rigid cell wall made of cellulose, and fungi one of chitin, so most viruses can only get inside the cells after trauma to the cell wall. However, nearly all plant viruses (such as tobacco mosaic virus) can also move directly from cell to cell, in the form of single-stranded nucleoprotein complexes, through pores

called plasmodesmata. This process requires movement proteins, which are virus-encoded proteins probably originally derived from plant proteins, which interact with the plasmodesmatal transport machinery Bacteria, like plants, have strong cell walls that a virus must breach to infect the cell. However, given that bacterial cell walls are much less thick than plant cell walls due to their much smaller size, some viruses have evolved mechanisms that inject their genome into the bacterial cell across the cell wall, while the viral capsid remains outside.

- *Uncoating* is a process in which the viral capsid is removed: this may be by degradation by viral enzymes or host enzymes or by simple dissociation; the end result is the releasing of the viral genomic nucleic acid.
- *Replication* of viruses primarily involves multiplication of the genome; however, this involves synthesis of viral messenger RNA (mRNA) for all viruses except some positive sense RNA viruses, from "early" genes; viral protein synthesis, possible assembly of viral proteins, then viral genome replication mediated by early or regulatory protein expression. This may be followed, for complex viruses with larger genomes, by one or more further rounds of mRNA synthesis: "late" gene expression is generally of structural or virion proteins.
- Following the structure-mediated self-*assembly* of the virus particles, some modification of the proteins often occurs. In viruses such as HIV, this modification (sometimes called maturation) occurs *after* the virus has been released from the host cell.
- Viruses can be *released* from the host cell by lysis, a process that kills the cell by bursting its membrane and cell wall if present: this is a feature of many bacterial and some animal viruses. Some viruses undergo a lysogenic cycle where the viral genome is incorporated by genetic recombination into a specific place in the host's chromosome. The viral genome is then known as a "provirus" or, in the case of bacteriophages a "prophage". Whenever the host divides, the viral genome is also replicated. The viral genome is mostly silent within the host; however, at some point, the provirus or prophage may give rise to active virus, which may lyse the host cells. Enveloped viruses (e.g., HIV) typically are released from the host cell by budding. During this process the virus acquires its envelope, which is a modified piece of the host's plasma or other, internal membrane.

The genetic material within virus particles, and the method by which the material is replicated, varies considerably between different types of viruses.

DNA viruses

The genome replication of most DNA viruses takes place in the cell's nucleus. If the cell has the appropriate receptor on its surface, these viruses enter the cell sometimes by direct fusion with the cell membrane (e.g.: herpesviruses) or – more usually – by receptor-mediated endocytosis. Most DNA viruses are entirely dependent on the host cell's DNA and RNA synthesising machinery, and RNA processing machinery; however, viruses with larger genomes may encode much of this machinery themselves. In eukaryotes the viral genome must cross the cell's nuclear membrane to access this machinery, while in bacteria it need only enter the cell.

RNA viruses
: Replication usually takes place in the cytoplasm. RNA viruses can be placed into four different groups depending on their modes of replication. The polarity (whether or not it can be used directly by ribosomes to make proteins) of single-stranded RNA viruses largely determines the replicative mechanism; the other major criterion is whether the genetic material is single-stranded or double-stranded. All RNA viruses use their own RNA replicase enzymes to create copies of their genomes.

Reverse transcribing viruses
: These have ssRNA (*Retroviridae*, *Metaviridae*, *Pseudoviridae*) or dsDNA (*Caulimoviridae*, and *Hepadnaviridae*) in their particles. Reverse transcribing viruses with RNA genomes (retroviruses), use a DNA intermediate to replicate, whereas those with DNA genomes (pararetroviruses) use an RNA intermediate during genome replication. Both types use a reverse transcriptase, or RNA-dependent DNA polymerase enzyme, to carry out the nucleic acid conversion. Retroviruses integrate the DNA produced by reverse transcription into the host genome as a provirus as a part of the replication process; pararetroviruses do not, although integrated genome copies of especially plant pararetroviruses can give rise to infectious virus. They are susceptible to antiviral drugs that inhibit the reverse transcriptase enzyme, e.g. zidovudine and lamivudine. An example of the first type is HIV, which is a retrovirus. Examples of the second type are the *Hepadnaviridae*, which includes Hepatitis B virus.

Effects on the host cell

The range of structural and biochemical effects that viruses have on the host cell is extensive. These are called *cytopathic effects*. Most virus infections eventually result in the death of the host cell. The causes of death include cell lysis, alterations to the cell's surface membrane and apoptosis. Often cell death is caused by cessation of its normal activities because of suppression by virus-specific proteins, not all of which are components of the virus particle.

Some viruses cause no apparent changes to the infected cell. Cells in which the virus is latent and inactive show few signs of infection and often function normally. This causes persistent infections and the virus is often dormant for many months or years. This is often the case with herpes viruses. Some viruses, such as Epstein-Barr virus, can cause cells to proliferate without causing malignancy, while others, such as papillomaviruses, are established causes of cancer.

Host range

Viruses are by far the most abundant parasites on earth and they have been found to infect all types of cellular life including animals, plants and bacteria. However, different types of viruses can only infect a limited range of hosts and many are species-specific. Some, such as smallpox virus for example, can only infect one species – in this case humans, and are said to have a narrow host range. Other viruses, such as rabies virus, can

infect different species of mammals and are said to have a broad range. The viruses that infect plants are harmless to animals and most viruses that infect other animals are harmless to humans. The host range of some bacteriophages is limited to a single strain of bacteria and they can be used to trace the source of outbreaks of infections by a method called phage typing.

Classification

Classification seeks to describe the diversity of viruses by naming and grouping them on the basis of similarities. In 1962, André Lwoff, Robert Horne, and Paul Tournier were the first to develop a means of virus classification, based on the Linnaean hierarchical system. This system bases classification on phylum, class, order, family, genus, and species. Viruses were grouped according to their shared properties (not those of their hosts) and the type of nucleic acid forming their genomes. Later the International Committee on Taxonomy of Viruses was formed. However, viruses are not classified on the basis of phylum or class, as their small genome size and high rate of mutation makes it difficult to determine their ancestry beyond Order. As such, the Baltimore Classification is used to supplement the more traditional hierarchy.

ICTV classification

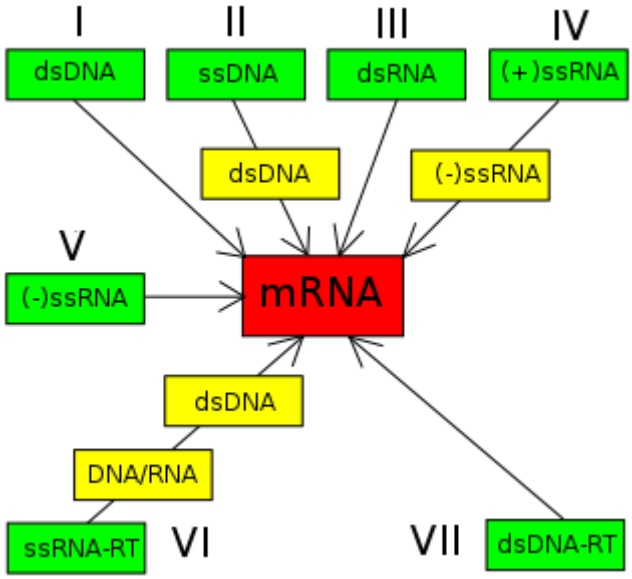
The International Committee on Taxonomy of Viruses (ICTV) developed the current classification system and wrote guidelines that put a greater weight on certain virus properties to maintain family uniformity. A unified taxonomy (a universal system for classifying viruses) has been established. The 7th lCTV Report formalised for the first time the concept of the virus species as the lowest taxon (group) in a branching hierarchy of viral taxa. However, at present only a small part of the total diversity of viruses has been studied, with analyses of samples from humans finding that about 20% of the virus sequences recovered have not been seen before, and samples from the environment, such as from seawater and ocean sediments, finding that the large majority of sequences are completely novel.

The general taxonomic structure is as follows:

Order (-virales)
Family (-viridae)
Subfamily (-virinae)
Genus (-*virus*)
Species (-*virus*)

In the current (2008) ICTV taxonomy, five orders have been established, the Caudovirales, Herpesvirales, Mononegavirales, Nidovirales, and Picornavirales. The committee does not formally distinguish between subspecies, strains, and isolates. In total there are 5 orders, 82 families, 11 subfamilies, 307 genera, 2,083 species and about 3,000 types yet unclassified.

Baltimore classification



The Baltimore Classification of viruses is based on the method of viral mRNA synthesis

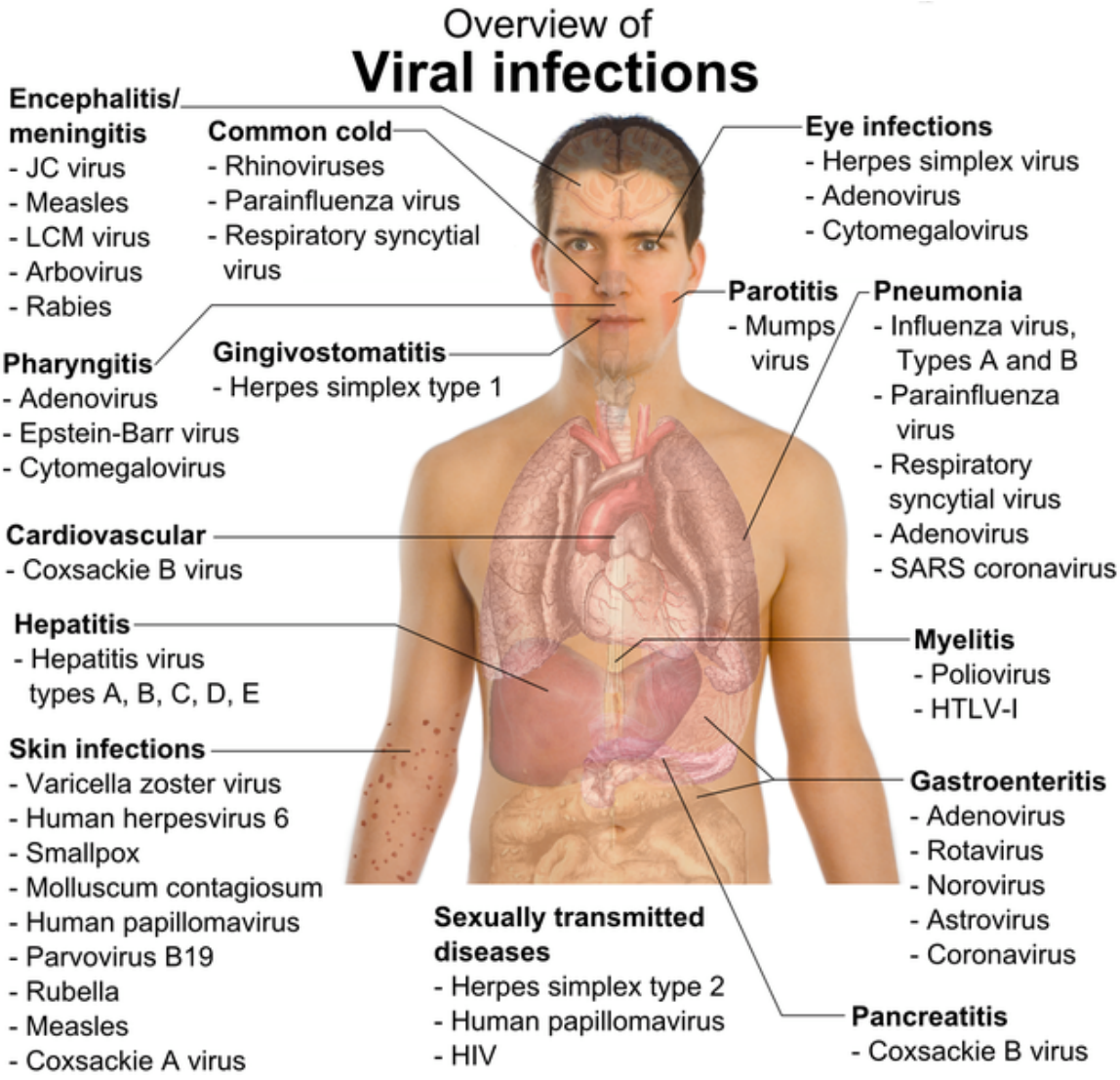
The Nobel Prize-winning biologist David Baltimore devised the Baltimore classification system. The ICTV classification system is used in conjunction with the Baltimore classification system in modern virus classification.

The Baltimore classification of viruses is based on the mechanism of mRNA production. Viruses must generate mRNAs from their genomes to produce proteins and replicate themselves, but different mechanisms are used to achieve this in each virus family. Viral genomes may be single-stranded (ss) or double-stranded (ds), RNA or DNA, and may or may not use reverse transcriptase (RT). Additionally, ssRNA viruses may be either sense (+) or antisense (−). This classification places viruses into seven groups:

- I: **dsDNA viruses** (e.g. Adenoviruses, Herpesviruses, Poxviruses)
- II: **ssDNA viruses** (+)sense DNA (e.g. Parvoviruses)
- III: **dsRNA viruses** (e.g. Reoviruses)
- IV: **(+)ssRNA viruses** (+)sense RNA (e.g. Picornaviruses, Togaviruses)
- V: **(−)ssRNA viruses** (−)sense RNA (e.g. Orthomyxoviruses, Rhabdoviruses)
- VI: **ssRNA-RT viruses** (+)sense RNA with DNA intermediate in life-cycle (e.g. Retroviruses)
- VII: **dsDNA-RT viruses** (e.g. Hepadnaviruses)

As an example of viral classification, the chicken pox virus, varicella zoster (VZV), belongs to the order Herpesvirales, family *Herpesviridae*, subfamily *Alphaherpesvirinae*, and genus *Varicellovirus*. VZV is in Group I of the Baltimore Classification because it is a dsDNA virus that does not use reverse transcriptase.

Viruses and human disease



Overview of the main types of viral infection and the most notable species involved

Examples of common human diseases caused by viruses include the common cold, influenza, chickenpox and cold sores. Many serious diseases such as ebola, AIDS, avian influenza and SARS are caused by viruses. The relative ability of viruses to cause disease is described in terms of virulence. Other diseases are under investigation as to whether they too have a virus as the causative agent, such as the possible connection between human herpes virus six (HHV6) and neurological diseases such as multiple sclerosis and chronic fatigue syndrome. There is controversy over whether the borna virus, previously thought to cause neurological diseases in horses, could be responsible for psychiatric illnesses in humans.

Viruses have different mechanisms by which they produce disease in an organism, which largely depends on the viral species. Mechanisms at the cellular level primarily include cell lysis, the breaking open and subsequent death of the cell. In multicellular organisms, if enough cells die the whole organism will start to suffer the effects. Although viruses cause disruption of healthy homeostasis, resulting in disease, they may exist relatively harmlessly within an organism. An example would include the ability of the herpes simplex virus, which causes cold sores, to remain in a dormant state within the human body. This is called latency and is a characteristic of the herpes viruses including Epstein-Barr virus, which causes glandular fever, and varicella zoster virus, which causes chickenpox and shingles. Most people have been infected with at least one of these types of herpes virus. However, these latent viruses might sometimes be beneficial, as the presence of the virus can increase immunity against bacterial pathogens, such as *Yersinia pestis*.

Some viruses can cause life-long or chronic infections, where the viruses continue to replicate in the body despite the host's defence mechanisms. This is common in hepatitis B virus and hepatitis C virus infections. People chronically infected are known as carriers, as they serve as reservoirs of infectious virus. In populations with a high proportion of carriers, the disease is said to be endemic.

Epidemiology

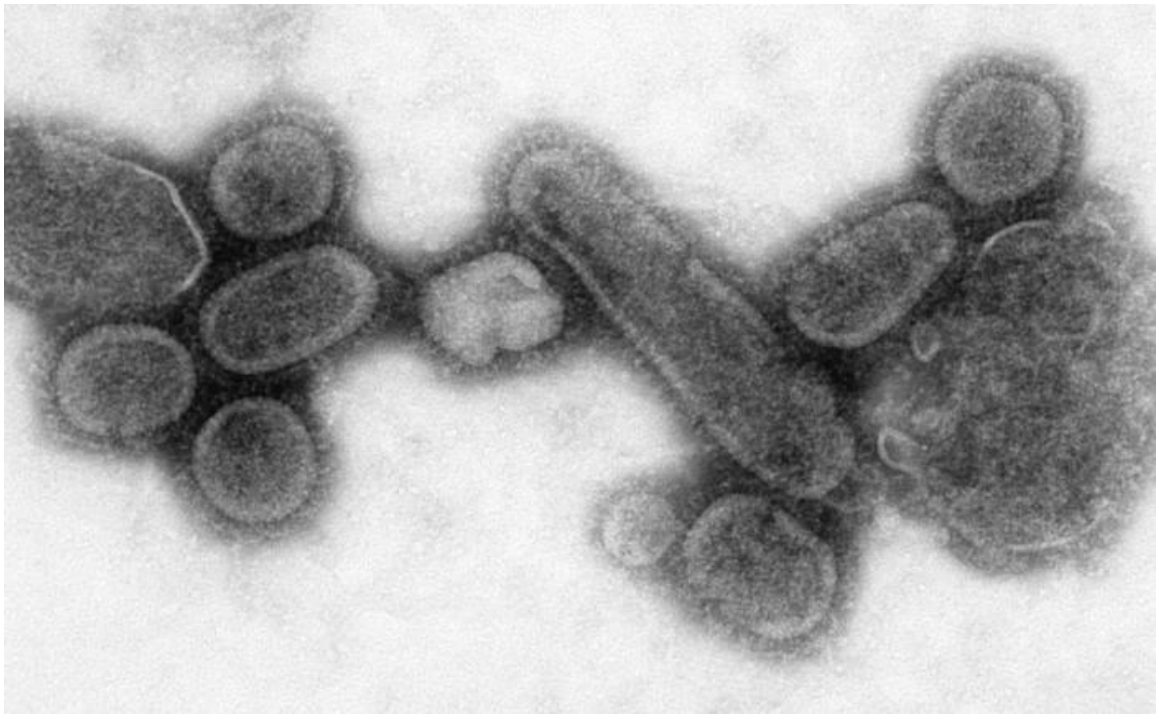
Viral epidemiology is the branch of medical science that deals with the transmission and control of virus infections in humans. Transmission of viruses can be vertical, that is from mother to child, or horizontal, which means from person to person. Examples of vertical transmission include hepatitis B virus and HIV where the baby is born already infected with the virus. Another, more rare, example is the varicella zoster virus, which although causing relatively mild infections in humans, can be fatal to the foetus and newly born baby.

Horizontal transmission is the most common mechanism of spread of viruses in populations. Transmission can occur when: body fluids are exchanged during sexual activity, e.g. HIV; blood is exchanged by contaminated transfusion or needle sharing, e.g. hepatitis C; a child is born to an infected mother, e.g. hepatitis B; exchange of saliva by mouth, e.g. Epstein-Barr virus; contaminated food or water is ingested, e.g. norovirus; aerosols containing virions are inhaled, e.g. influenza virus; and insect vectors such as mosquitoes penetrate the skin of a host, e.g. dengue. The rate or speed of transmission of viral infections depends on factors that include population density, the number of susceptible individuals, (i.e. those who are not immune), the quality of health care and the weather.

Epidemiology is used to break the chain of infection in populations during outbreaks of viral diseases. Control measures are used that are based on knowledge of how the virus is transmitted. It is important to find the source, or sources, of the outbreak and to identify the virus. Once the virus has been identified, the chain of transmission can sometimes be broken by vaccines. When vaccines are not available sanitation and disinfection can be

effective. Often infected people are isolated from the rest of the community and those that have been exposed to the virus placed in quarantine. To control the outbreak of foot and mouth disease in cattle in Britain in 2001, thousands of cattle were slaughtered. Most viral infections of humans and other animals have incubation periods during which the infection causes no signs or symptoms. Incubation periods for viral diseases range from a few days to weeks but are known for most infections. Somewhat overlapping, but mainly following the incubation period, there is a period of communicability; a time when an infected individual or animal is contagious and can infect another person or animal. This too is known for many viral infections and knowledge the length of both periods is important in the control of outbreaks. When outbreaks cause an unusually high proportion of cases in a population, community or region they are called epidemics. If outbreaks spread worldwide they are called pandemics.

Epidemics and pandemics



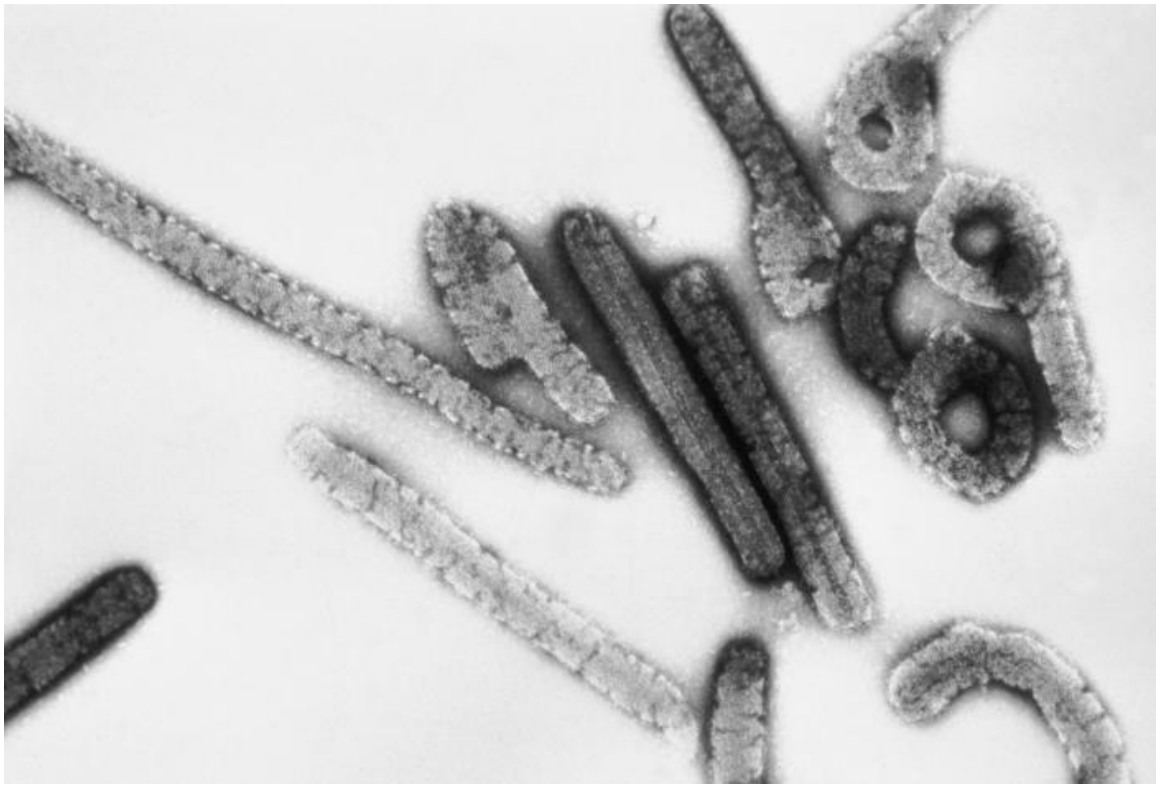
Transmission electron microscope image of a recreated 1918 influenza virus

Native American populations were devastated by contagious diseases, particularly smallpox, brought to the Americas by European colonists. It is unclear how many Native Americans were killed by foreign diseases after the arrival of Columbus in the Americas, but the numbers have been estimated to be close to 70% of the indigenous population. The damage done by this disease significantly aided European attempts to displace and conquer the native population.

A pandemic is a worldwide epidemic. The 1918 flu pandemic, commonly referred to as the Spanish flu, was a category 5 influenza pandemic caused by an unusually severe and

deadly influenza A virus. The victims were often healthy young adults, in contrast to most influenza outbreaks, which predominantly affect juvenile, elderly, or otherwise weakened patients.

The Spanish flu pandemic lasted from 1918 to 1919. Older estimates say it killed 40–50 million people, while more recent research suggests that it may have killed as many as 100 million people, or 5% of the world's population in 1918. Most researchers believe that HIV originated in sub-Saharan Africa during the 20th century; it is now a pandemic, with an estimated 38.6 million people now living with the disease worldwide. The Joint United Nations Programme on HIV/AIDS (UNAIDS) and the World Health Organization (WHO) estimate that AIDS has killed more than 25 million people since it was first recognised on June 5, 1981, making it one of the most destructive epidemics in recorded history. In 2007 there were 2.7 million new HIV infections and 2 million HIV-related deaths.



Marburg virus

Several highly lethal viral pathogens are members of the *Filoviridae*. Filoviruses are filament-like viruses that cause viral hemorrhagic fever, and include the ebola and marburg viruses. The Marburg virus attracted widespread press attention in April 2005 for an outbreak in Angola. Beginning in October 2004 and continuing into 2005, the outbreak was the world's worst epidemic of any kind of viral hemorrhagic fever.

Cancer

Viruses are an established cause of cancer in humans and other species. Viral cancers only occur in a minority of infected persons (or animals). Cancer viruses come from a range of virus families, including both RNA and DNA viruses, and so there is no single type of "oncovirus" (an obsolete term originally used for acutely transforming retroviruses). The development of cancer is determined by a variety of factors such as host immunity and mutations in the host. Viruses accepted to cause human cancers include some genotypes of human papillomavirus, hepatitis B virus, hepatitis C virus, Epstein-Barr virus, Kaposi's sarcoma-associated herpesvirus and human T-lymphotropic virus. The most recently discovered human cancer virus is a polyomavirus (Merkel cell polyomavirus) that causes most cases of a rare form of skin cancer called Merkel cell carcinoma. Hepatitis viruses can develop into a chronic viral infection that leads to liver cancer. Infection by human T-lymphotropic virus can lead to tropical spastic paraparesis and adult T-cell leukemia. Human papillomaviruses are an established cause of cancers of cervix, skin, anus, and penis. Within the *Herpesviridae*, Kaposi's sarcoma-associated herpesvirus causes Kaposi's sarcoma and body cavity lymphoma, and Epstein–Barr virus causes Burkitt's lymphoma, Hodgkin's lymphoma, B lymphoproliferative disorder and nasopharyngeal carcinoma. Merkel cell polyomavirus closely related to SV40 and mouse polyomaviruses that have been used as animal models for cancer viruses for over 50 years.

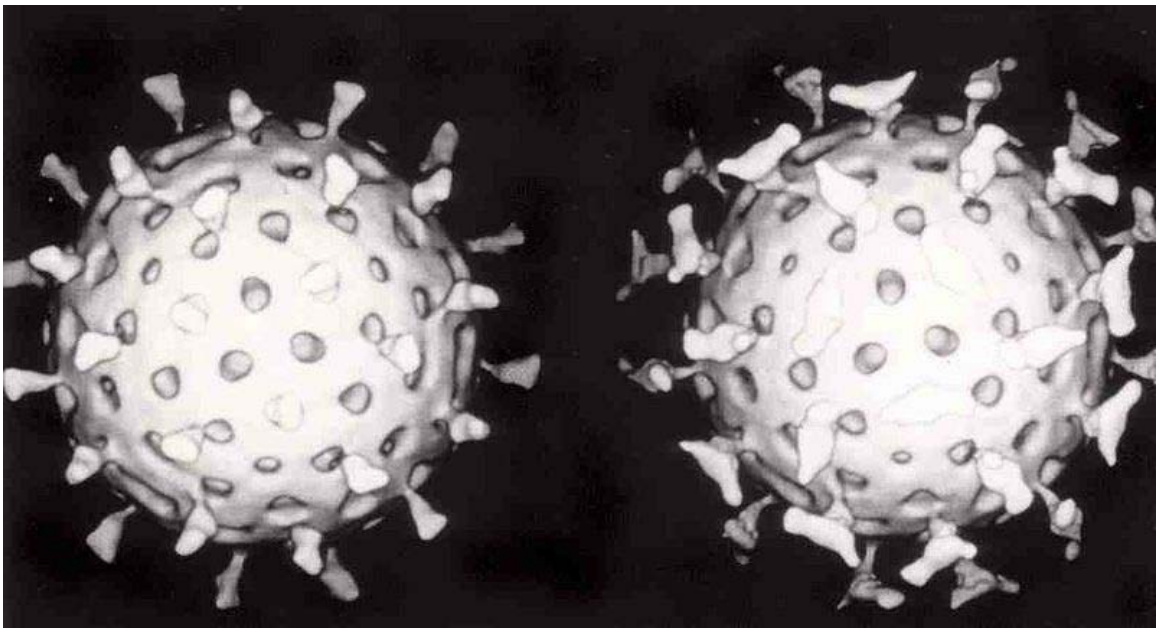
Host defence mechanisms

The body's first line of defence against viruses is the innate immune system. This comprises cells and other mechanisms that defend the host from infection in a non-specific manner. This means that the cells of the innate system recognise, and respond to, pathogens in a generic way, but unlike the adaptive immune system, it does not confer long-lasting or protective immunity to the host.

RNA interference is an important innate defence against viruses. Many viruses have a replication strategy that involves double-stranded RNA (dsRNA). When such a virus infects a cell, it releases its RNA molecule or molecules, which immediately bind to a protein complex called dicer that cuts the RNA into smaller pieces. A biochemical pathway called the RISC complex is activated, which degrades the viral mRNA and the cell survives the infection. Rotaviruses avoid this mechanism by not uncoating fully inside the cell and by releasing newly produced mRNA through pores in the particle's inner capsid. The genomic dsRNA remains protected inside the core of the virion.

When the adaptive immune system of a vertebrate encounters a virus, it produces specific antibodies that bind to the virus and render it non-infectious. This is called humoral immunity. Two types of antibodies are important. The first, called IgM, is highly effective at neutralizing viruses but is only produced by the cells of the immune system for a few weeks. The second, called IgG, is produced indefinitely. The presence of IgM in the blood of the host is used to test for acute infection, whereas IgG indicates an

infection sometime in the past. IgG antibody is measured when tests for immunity are carried out.



Two rotaviruses: the one on the right is coated with antibodies that stop its attaching to cells and infecting them

A second defence of vertebrates against viruses is called cell-mediated immunity and involves immune cells known as T cells. The body's cells constantly display short fragments of their proteins on the cell's surface, and if a T cell recognises a suspicious viral fragment there, the host cell is destroyed by *killer T* cells and the virus-specific T-cells proliferate. Cells such as the macrophage are specialists at this antigen presentation. The production of interferon is an important host defence mechanism. This is a hormone produced by the body when viruses are present. Its role in immunity is complex, but it eventually stops the viruses from reproducing by killing the infected cell and its close neighbours.

Not all virus infections produce a protective immune response in this way. HIV evades the immune system by constantly changing the amino acid sequence of the proteins on the surface of the virion. These persistent viruses evade immune control by sequestration, blockade of antigen presentation, cytokine resistance, evasion of natural killer cell activities, escape from apoptosis, and antigenic shift. Other viruses, called *neurotropic viruses*, are disseminated by neural spread where the immune system may be unable to reach them.

Prevention and treatment

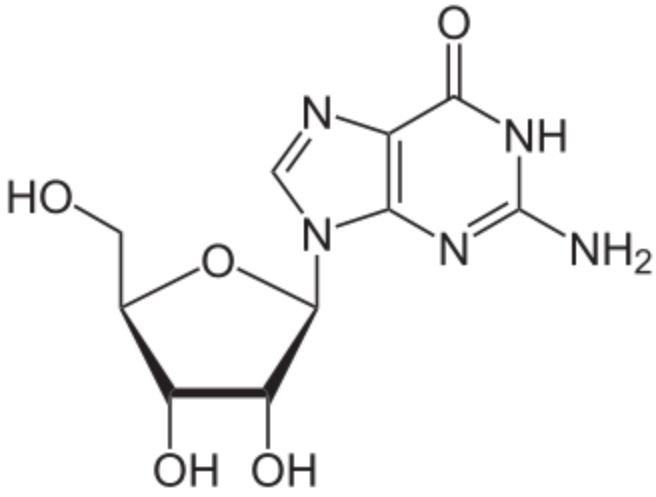
Because viruses use vital metabolic pathways within host cells to replicate, they are difficult to eliminate without using drugs that cause toxic effects to host cells in general.

The most effective medical approaches to viral diseases are vaccinations to provide immunity to infection, and antiviral drugs that selectively interfere with viral replication.

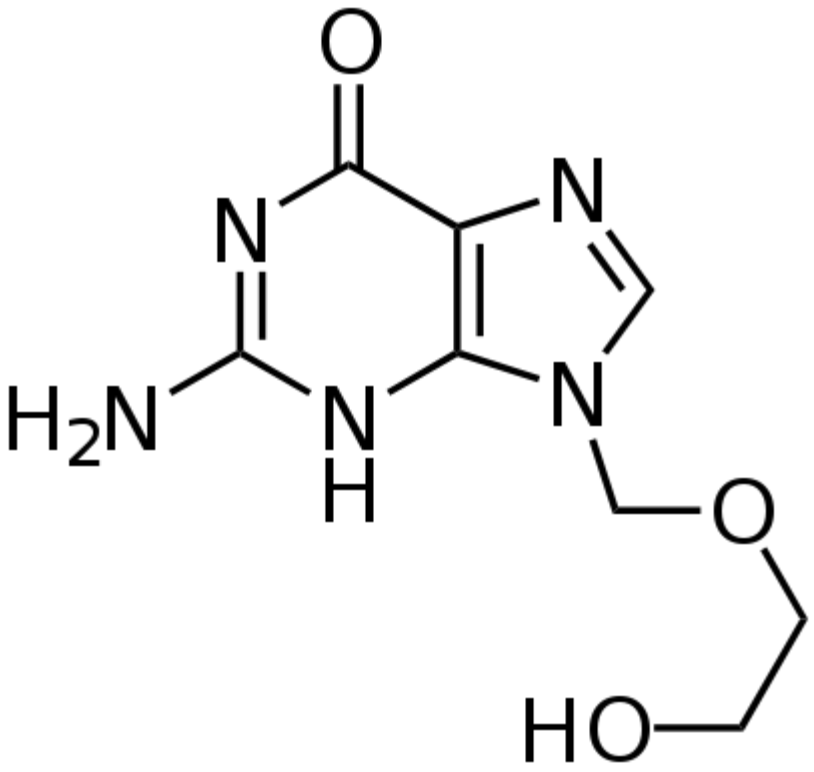
Vaccines

Vaccination is a cheap and effective way of preventing infections by viruses. Vaccines were used to prevent viral infections long before the discovery of the actual viruses. Their use has resulted in a dramatic decline in morbidity (illness) and mortality (death) associated with viral infections such as polio, measles, mumps and rubella. Smallpox infections have been eradicated. Vaccines are available to prevent over thirteen viral infections of humans, and more are used to prevent viral infections of animals. Vaccines can consist of live-attenuated or killed viruses, or viral proteins (antigens). Live vaccines contain weakened forms of the virus, which do not cause the disease but nonetheless confer immunity. Such viruses are called attenuated. Live vaccines can be dangerous when given to people with a weak immunity, (who are described as immunocompromised), because in these people, the weakened virus can cause the original disease. Biotechnology and genetic engineering techniques are used to produce subunit vaccines. These vaccines use only the capsid proteins of the virus. Hepatitis B vaccine is an example of this type of vaccine. Subunit vaccines are safe for immunocompromised patients because they cannot cause the disease. The yellow fever virus vaccine, a live-attenuated strain called 17D, is probably the safest and most effective vaccine ever generated.

Antiviral drugs



Guanosine



The guanosine analogue Aciclovir

Antiviral drugs are often nucleoside analogues, (fake DNA building blocks), which viruses mistakenly incorporate into their genomes during replication. The life-cycle of the virus is then halted because the newly synthesised DNA is inactive. This is because these analogues lack the hydroxyl groups, which, along with phosphorus atoms, link together to form the strong "backbone" of the DNA molecule. This is called DNA chain termination. Examples of nucleoside analogues are aciclovir for Herpes simplex virus infections and lamivudine for HIV and Hepatitis B virus infections. Aciclovir is one of the oldest and most frequently prescribed antiviral drugs. Other antiviral drugs in use target different stages of the viral life cycle. HIV is dependent on a proteolytic enzyme called the HIV-1 protease for it to become fully infectious. There is a large class of drugs called protease inhibitors that inactivate this enzyme.

Hepatitis C is caused by an RNA virus. In 80% of people infected, the disease is chronic, and without treatment, they are infected for the remainder of their lives. However, there is now an effective treatment that uses the nucleoside analogue drug ribavirin combined with interferon. The treatment of chronic carriers of the hepatitis B virus by using a similar strategy using lamivudine has been developed.

Infection in other species

Viruses infect all cellular life and, although viruses occur universally, each cellular species has its own specific range that often infect only that species. Some viruses, called satellites, can only replicate within cells that have already been infected by another virus. Viruses are important pathogens of livestock. Diseases such as Foot and Mouth Disease and bluetongue are caused by viruses. Companion animals such as cats, dogs, and horses, if not vaccinated, are susceptible to serious viral infections. Canine parvovirus is caused by a small DNA virus and infections are often fatal in pups. Like all invertebrates, the honey bee is susceptible to many viral infections. Fortunately, most viruses co-exist harmlessly in their host and cause no signs or symptoms of disease.

Plants

Peppers infected by mild mottle virus

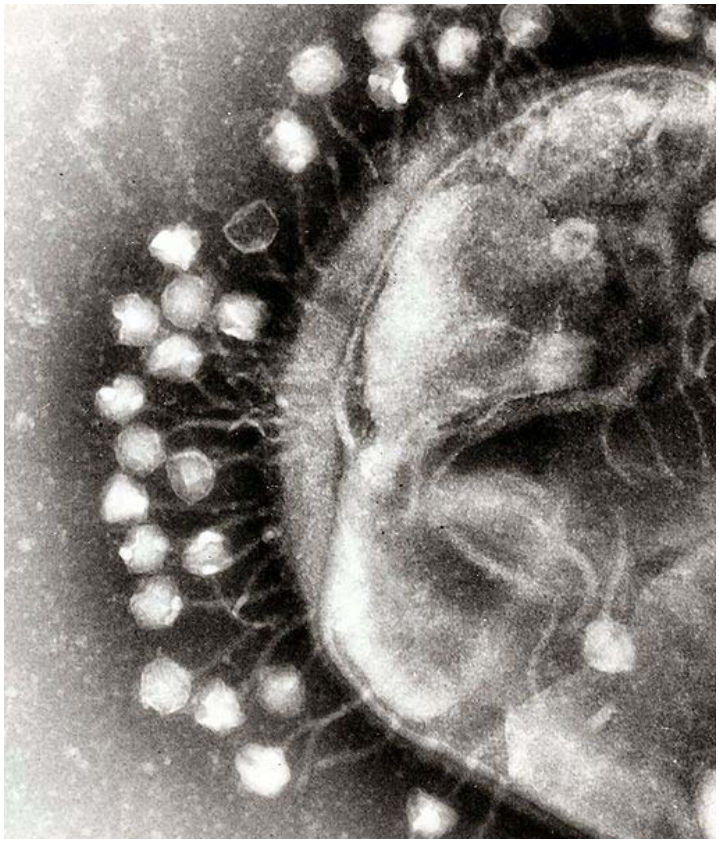
There are many types of plant virus, but often they cause only a loss of yield, and it is not economically viable to try to control them. Plant viruses are often spread from plant to plant by organisms, known as *vectors*. These are normally insects, but some fungi, nematode worms and single-celled organisms have been shown to be vectors. When control of plant virus infections is considered economical, for perennial fruits for

example, efforts are concentrated on killing the vectors and removing alternate hosts such as weeds. Plant viruses are harmless to humans and other animals because they can reproduce only in living plant cells.

Plants have elaborate and effective defence mechanisms against viruses. One of the most effective is the presence of so-called resistance (R) genes. Each R gene confers resistance to a particular virus by triggering localised areas of cell death around the infected cell, which can often be seen with the unaided eye as large spots. This stops the infection from spreading. RNA interference is also an effective defence in plants. When they are infected, plants often produce natural disinfectants that kill viruses, such as salicylic acid, nitric oxide, and reactive oxygen molecules.

Plant virus particles or virus-like particles (VLPs) have applications in both biotechnology and nanotechnology. The capsids of most plant viruses are simple and robust structures and can be produced in large quantities either by the infection of plants or by expression in a variety of heterologous systems. Plant virus particles can be modified genetically and chemically to encapsulate foreign material and can be incorporated into supramolecular structures for use in biotechnology.

Bacteria



Transmission electron micrograph of multiple bacteriophages attached to a bacterial cell wall

Bacteriophages are a common and diverse group of viruses and are the most abundant form of biological entity in aquatic environments – there are up to ten times more of these viruses in the oceans than there are bacteria, reaching levels of 250,000,000 bacteriophages per millilitre of seawater. These viruses infect specific bacteria by binding to surface receptor molecules and then entering the cell. Within a short amount of time, in some cases just minutes, bacterial polymerase starts translating viral mRNA into protein. These proteins go on to become either new virions within the cell, helper proteins, which help assembly of new virions, or proteins involved in cell lysis. Viral enzymes aid in the breakdown of the cell membrane, and, in the case of the T4 phage, in just over twenty minutes after injection over three hundred phages could be released.

The major way bacteria defend themselves from bacteriophages is by producing enzymes that destroy foreign DNA. These enzymes, called restriction endonucleases, cut up the viral DNA that bacteriophages inject into bacterial cells. Bacteria also contain a system that uses CRISPR sequences to retain fragments of the genomes of viruses that the bacteria have come into contact with in the past, which allows them to block the virus's replication through a form of RNA interference. This genetic system provides bacteria with acquired immunity to infection.

Archaea

Some viruses replicate within archaea: these are double-stranded DNA viruses with unusual and sometimes unique shapes. These viruses have been studied in most detail in the thermophilic archaea, particularly the orders Sulfolobales and Thermoproteales. Defences against these viruses may involve RNA interference from repetitive DNA sequences within archaean genomes that are related to the genes of the viruses.

Role in aquatic ecosystems

Viruses are the most abundant biological entity in aquatic environments: a teaspoon of seawater contains about one million of them. They are essential to the regulation of saltwater and freshwater ecosystems. Most of these viruses are bacteriophages, which are harmless to plants and animals. They infect and destroy the bacteria in aquatic microbial communities, comprising the most important mechanism of recycling carbon in the marine environment. The organic molecules released from the bacterial cells by the viruses stimulates fresh bacterial and algal growth.

Microorganisms constitute more than 90% of the biomass in the sea. It is estimated that viruses kill approximately 20% of this biomass each day and that there are fifteen times as many viruses in the oceans as there are bacteria and archaea. Viruses are the main agents responsible for the rapid destruction of harmful algal blooms, which often kill other marine life. The number of viruses in the oceans decreases further offshore and deeper into the water, where there are fewer host organisms.

The effects of marine viruses are far-reaching; by increasing the amount of photosynthesis in the oceans, viruses are indirectly responsible for reducing the amount of carbon dioxide in the atmosphere by approximately 3 gigatonnes of carbon per year.

Like any organism, marine mammals are susceptible to viral infections. In 1988 and 2002 thousands of harbour seals were killed in Europe by phocine distemper virus. Many other viruses, including caliciviruses, herpesviruses, adenoviruses and parvoviruses, circulate in marine mammal populations.

Role in evolution

Viruses are an important natural means of transferring genes between different species, which increases genetic diversity and drives evolution. It is thought that viruses played a central role in the early evolution, before the diversification of bacteria, archaea and eukaryotes and at the time of the last universal common ancestor of life on Earth. Viruses are still one of the largest reservoirs of unexplored genetic diversity on the Earth.

Applications

Life sciences and medicine

Scientist studying the H5N1 influenza virus

Viruses are important to the study of molecular and cellular biology as they provide simple systems that can be used to manipulate and investigate the functions of cells. The study and use of viruses have provided valuable information about aspects of cell biology. For example, viruses have been useful in the study of genetics and helped our understanding of the basic mechanisms of molecular genetics, such as DNA replication, transcription, RNA processing, translation, protein transport, and immunology.

Geneticists often use viruses as vectors to introduce genes into cells that they are studying. This is useful for making the cell produce a foreign substance, or to study the effect of introducing a new gene into the genome. In similar fashion, virotherapy uses viruses as vectors to treat various diseases, as they can specifically target cells and DNA. It shows promising use in the treatment of cancer and in gene therapy. Eastern European scientists have used phage therapy as an alternative to antibiotics for some time, and interest in this approach is increasing, because of the high level of antibiotic resistance now found in some pathogenic bacteria.

Expression of heterologous proteins by viruses is the basis of several manufacturing processes that are currently being used for the production of various proteins such as vaccine antigens and antibodies. Industrial processes have been recently developed using viral vectors and a number of pharmaceutical proteins are currently in pre-clinical and clinical trials.

Materials science and nanotechnology

Current trends in nanotechnology promise to make much more versatile use of viruses. From the viewpoint of a materials scientist, viruses can be regarded as organic nanoparticles. Their surface carries specific tools designed to cross the barriers of their host cells. The size and shape of viruses, and the number and nature of the functional groups on their surface, is precisely defined. As such, viruses are commonly used in materials science as scaffolds for covalently linked surface modifications. A particular quality of viruses is that they can be tailored by directed evolution. The powerful techniques developed by life sciences are becoming the basis of engineering approaches towards nanomaterials, opening a wide range of applications far beyond biology and medicine.

Because of their size, shape, and well-defined chemical structures, viruses have been used as templates for organizing materials on the nanoscale. Recent examples include work at the Naval Research Laboratory in Washington, DC, using Cowpea Mosaic Virus (CPMV) particles to amplify signals in DNA microarray based sensors. In this application, the virus particles separate the fluorescent dyes used for signalling to prevent the formation of non-fluorescent dimers that act as quenchers. Another example is the use of CPMV as a nanoscale breadboard for molecular electronics.

Synthetic viruses

Many viruses can be synthesized de novo ("from scratch") and the first synthetic virus was created in 2002. Although somewhat of a misconception, it is not the actual virus that is synthesized, but rather its DNA genome (in case of a DNA virus), or a cDNA copy of its genome (in case of RNA viruses). For many virus families the naked synthetic DNA or RNA (once enzymatically converted back from the synthetic cDNA) is infectious when introduced into a cell. That is, they contain all the necessary information to produce new viruses. This technology is now being used to investigate novel vaccine strategies. The ability to synthesize viruses has far-reaching consequences, since viruses can no longer be regarded as extinct, as long as the information of their genome sequence is known and permissive cells are available. Currently, the full-length genome sequences of 2408 different viruses (including smallpox) are publicly available at an online database, maintained by the National Institute of Health.

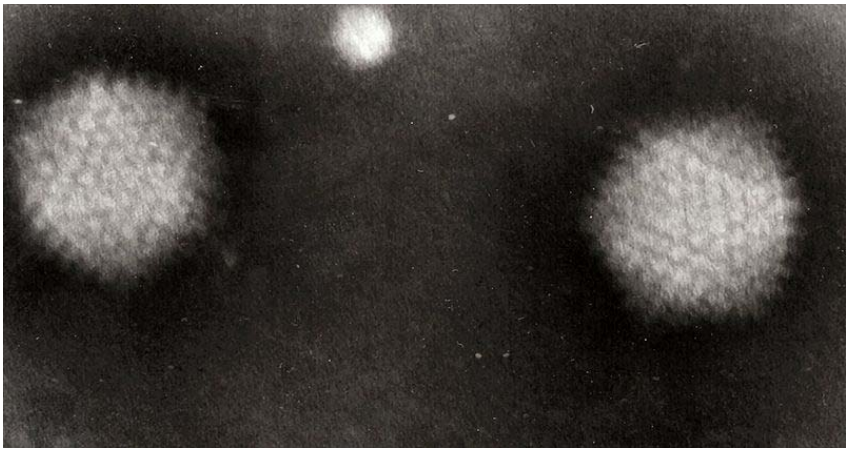
Weapons

The ability of viruses to cause devastating epidemics in human societies has led to the concern that viruses could be weaponised for biological warfare. Further concern was raised by the successful recreation of the infamous 1918 influenza virus in a laboratory. The smallpox virus devastated numerous societies throughout history before its eradication. There are officially only two centers in the world which keep stocks of smallpox virus – the Russian Vector laboratory, and the United States Centers for Disease Control. But fears that it may be used as a weapon are not totally unfounded; the vaccine for smallpox has sometimes severe side-effects – during the last years before the eradication of smallpox disease more people became seriously ill as a result of vaccination than did people from smallpox — and smallpox vaccination is no longer universally practiced. Thus, much of the modern human population has almost no established resistance to smallpox.

Chapter 2

Adenoviridae

Adenoviruses



Transmission electron micrograph of two adenovirus particles

Virus classification	
Group:	Group I (dsDNA)
Family:	***Adenoviridae***

Genera
Atadenovirus
Aviadenovirus
Ichtadenovirus
Mastadenovirus
Siadenovirus

Adenoviruses are medium-sized (90–100 nm), nonenveloped (naked) icosahedral viruses composed of a nucleocapsid and a double-stranded linear DNA genome. There are 55 described serotypes in humans, which are responsible for 5–10% of upper respiratory infections in children, and many infections in adults as well.

Viruses of the family *Adenoviridae* infect various species of vertebrates, including humans. Adenoviruses were first isolated in human adenoids, from which the name is derived, and are classified as group I under the Baltimore classification scheme.

Virology

Classification

This family contains the following genera:

- Genus *Atadenovirus*; type species: *Ovine adenovirus D*
- Genus *Aviadenovirus*; type species: *Fowl adenovirus A*
- Genus *Ichtadenovirus*; type species: *Sturgeon adenovirus A*
- Genus *Mastadenovirus*; type species: *Human adenovirus C*; others include AD-36
- Genus *Siadenovirus*; type species: *Frog adenovirus*

Diversity

Classification of Adenoviridae can be complex.

In humans, there are 56 accepted human adenovirus types (HAdV-1 to 56) in seven species (Human adenovirus A to G):

- A: 12, 18, 31
- B: 3, 7, 11, 14, 16, 21, 34, 35, 50, 55
- C: 1, 2, 5, 6
- D: 8, 9, 10, 13, 15, 17, 19, 20, 22, 23, 24, 25, 26, 27, 28, 29, 30, 32, 33, 36, 37, 38, 39, 42, 43, 44, 45, 46, 47, 48, 49, 51, 53, 54, 56
- E: 4
- F: 40, 41
- G: 52

Different types/serotypes are associated with different conditions:

- respiratory disease (mainly species HAdV-B and C)
- conjunctivitis (HAdV-B and D)
- gastroenteritis (HAdV-F types 40, 41, HAdV-G type 52)

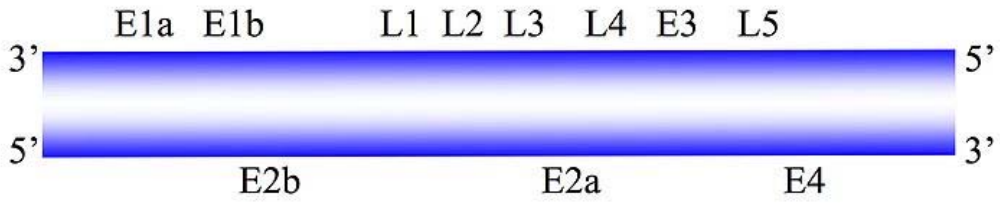
When not restricting the subject to human viruses, Adenoviridae can be divided into five genera: *Mastadenovirus*, *Aviadenovirus*, *Atadenovirus*, *Siadenovirus* and *Ichtadenovirus*.

Structure

Adenoviruses represent the largest nonenveloped viruses. Because of their large size, they are able to be transported through the endosome (i.e. envelope fusion is not necessary). The virion also has a unique "spike" or fiber associated with each penton base of the capsid that aids in attachment to the host cell via the coxsackie-adenovirus receptor on the surface of the host cell.

In 2010 scientists announced that they had solved the structure of the human adenovirus at the atomic level, making the largest high resolution model ever. The virus is composed of around 1 million amino acid residues and weighs around 150 MDa.

Genome

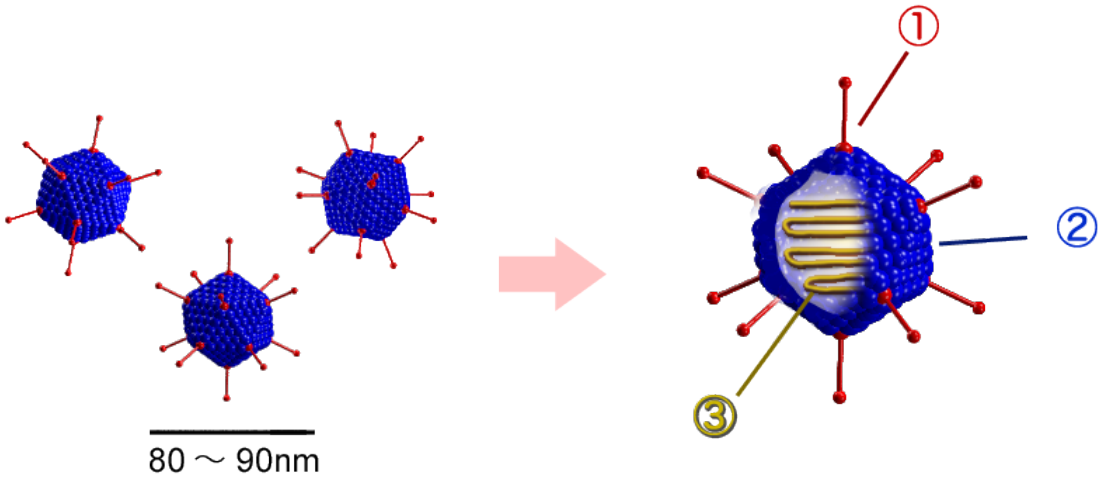


Schematic diagram of the linear adenovirus genome, showing Early genes (E) and Late genes (L).

The adenovirus genome is linear, non-segmented double stranded (ds) DNA which is between 26 and 45 Kbp. This allows the virus to theoretically carry 22 to 40 genes. Although this is significantly larger than other viruses in its Baltimore group it is still a very simple virus and is heavily reliant on the host cell for survival and replication. An interesting feature of this viral genome is that it has a terminal 55 kDa protein associated with each of the 5' ends of the linear dsDNA, these are used as primers in viral replication and ensure that the ends of the virus' linear genome are adequately replicated.

Replication

Adenoviruses possess a linear dsDNA genome and are able to replicate in the nucleus of mammalian cells using the host's replication machinery.



The structure of adenovirus. 1 = penton capsomeres 2 = hexon capsomeres, and 3= viral genome (linear dsDNA)

Entry of adenoviruses into the host cell involves two sets of interactions between the virus and the host cell. Entry into the host cell is initiated by the knob domain of the fiber protein binding to the cell receptor. The two currently established receptors are: CD46 for the group B human adenovirus serotypes and the coxsackievirus adenovirus receptor (CAR) for all other serotypes. There are some reports suggesting MHC molecules and sialic acid residues functioning in this capacity as well. This is followed by a secondary interaction, where a specialized motif in the penton base protein interacts with an integrin molecule. It is the co-receptor interaction that stimulates internalization of the adenovirus. This co-receptor molecule is αv integrin. Binding to αv integrin results in endocytosis of the virus particle via clathrin-coated pits. Attachment to αv integrin stimulates cell signaling and thus induces actin polymerization resulting in entry of the virion into the host cell within an endosome.

Once the virus has successfully gained entry into the host cell, the endosome acidifies, which alters virus topology by causing capsid components to disassociate. These changes as well as the toxic nature of the pentons results in the release of the virion into the cytoplasm. With the help of cellular microtubules the virus is transported to the nuclear pore complex whereby the adenovirus particle disassembles. Viral DNA is subsequently released which can enter the nucleus via the nuclear pore. After this the DNA associates with histone molecules. Thus viral gene expression can occur and new virus particles can be generated.

The adenovirus life cycle is separated, by the DNA replication process, into two phases: an early and a late phase. In both phases a primary transcript is generated which is alternatively spliced to generate monocistronic mRNAs compatible with the host's ribosome, allowing for the products to be translated.

The early genes are responsible for expressing mainly non-structural, regulatory proteins. The goal of these proteins is threefold: to alter the expression of host proteins that are necessary for DNA synthesis; to activate other virus genes (such as the virus-encoded DNA polymerase); and to avoid premature death of the infected cell by the host-immune defenses (blockage of apoptosis, blockage of interferon activity, and blockage of MHC class I translocation and expression).

Some adenoviruses under specialized conditions can transform cells using their early gene products. E1a (binds Retinoblastoma tumor suppressor protein) has been found to immortalize primary cells *in vitro* allowing E1b (binds p53 tumor suppressor) to assist and stably transform the cells. Nevertheless, they are reliant upon each other to successfully transform the host cell and form tumors.

DNA replication separates the early and late phases. Once the early genes have liberated adequate virus proteins, replication machinery and replication substrates, replication of the adenovirus genome can occur. A terminal protein that is covalently bound to the 5' end of the adenovirus genome acts as a primer for replication. The viral DNA polymerase then uses a strand displacement mechanism, as opposed to the conventional Okazaki fragments used in mammalian DNA replication, to replicate the genome.

The late phase of the adenovirus life cycle is focused on producing sufficient quantities of structural protein to pack all the genetic material produced by DNA replication. Once the viral components have successfully been replicated the virus is assembled into its protein shells and released from the cell as a result of virally induced cell lysis.

Epidemiology

Transmission

Adenoviruses are unusually stable to chemical or physical agents and adverse pH conditions, allowing for prolonged survival outside of the body and water. Adenoviruses are primarily spread via respiratory droplets, however they can also be spread by fecal routes as well.

Animals

Two types of canine adenoviruses are well known, type 1 and 2. Type 1 causes infectious canine hepatitis, a potentially fatal disease involving vasculitis and hepatitis. Type 1 infection can also cause respiratory and eye infections. *Canine adenovirus 2* (CAdV-2) is one of the potential causes of kennel cough. Core vaccines for dogs include attenuated

live CAdV-2, which produces immunity to CAdV-1 and CAdV-2. CAdV-1 was initially used in a vaccine for dogs, but corneal edema was a common complication.

Adenovirus in Reptiles has little known about it, but research is currently in progress.

Adenoviruses are also known to cause respiratory infections in horses, cattle, pigs, sheep, and goats. *Equine adenovirus 1* can also cause fatal disease in immunocompromised Arabian foals, involving pneumonia and destruction of pancreatic and salivary gland tissue.

Prevention

In the past, US military recruits were vaccinated against two serotypes of adenotypes, with a corresponding decrease in illnesses caused by those serotypes. The vaccine is no longer manufactured, and there are currently no vaccines available to protect against the adenovirus.

Good hygiene, including handwashing, is still the best way to avoid picking up the adenovirus from an infected person.

Infections

Most infections with adenovirus result in infections of the upper respiratory tract. Adenovirus infections often show up as conjunctivitis, tonsilitis (which may look exactly like strep throat and cannot be distinguished from strep except by throat culture), an ear infection, or croup. Adenoviruses can also cause gastroenteritis (stomach flu). A combination of conjunctivitis and tonsilitis is particularly common with adenovirus infections. Some children (especially small ones) can develop adenovirus bronchiolitis or pneumonia, both of which can be severe. In babies, adenoviruses can also cause coughing fits that look almost exactly like whooping cough. Adenoviruses can also cause viral meningitis or encephalitis. Rarely, adenovirus can cause hemorrhagic cystitis (inflammation of the urinary bladder—a form of urinary tract infection—with blood in the urine).

Most people recover from adenovirus infections by themselves, but people with immunodeficiency sometimes die of adenovirus infections, and—rarely—even previously healthy people can die of these infections.

Adenoviruses are often transmitted by coughed-out droplets, but can also be transmitted by contact with an infected person, or by virus particles left on objects such as towels and faucet handles. Some people with adenovirus gastroenteritis may shed the virus in their stools for months after getting over the symptoms. The virus can be passed from one person to another through some sexual practices, and through water in swimming pools that do not have enough chlorine in them. As with many other illnesses, good

handwashing is one way to lessen the spread of adenoviruses from one person to another. Heat and bleach will kill adenoviruses on objects.

Treatment

There are no antiviral drugs to treat adenoviral infections, so treatment is largely directed at the symptoms (such as acetaminophen for fever). A doctor may give antibiotic eyedrops for conjunctivitis, since it takes a while to test to see if the eye infection is bacterial or viral and to help prevent secondary bacterial infections.

Utilization in treatment of unrelated diseases

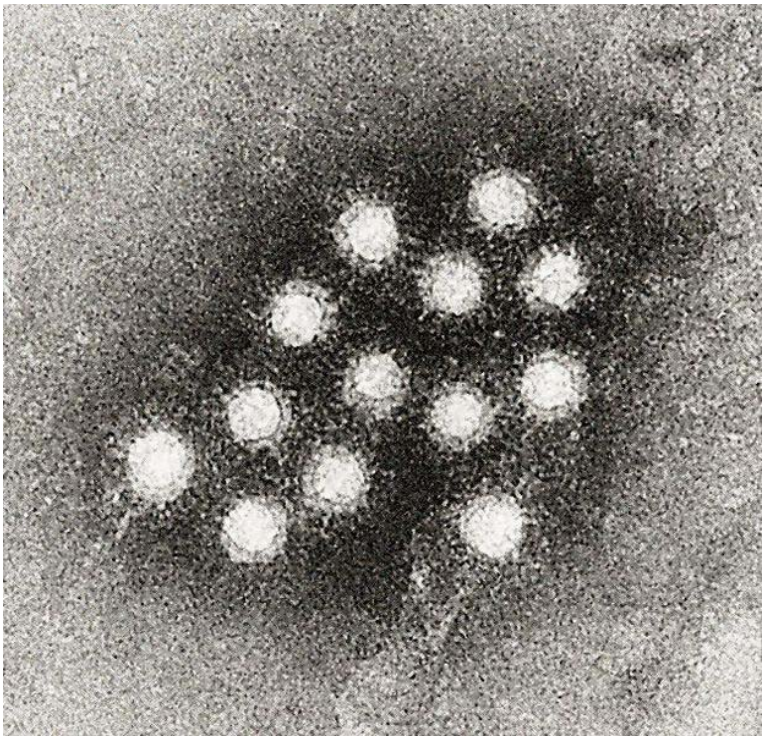
Adenovirus is used as a vehicle to administer targeted therapy, in the form of recombinant DNA or protein. Specific modifications on fibre proteins are used to target Adenovirus to certain cell types; a major effort is made to limit hepatotoxicity and prevent multiple organ failure. Adenovirus dodecahedron can qualify as a potent delivery platform for foreign antigens to human myeloid dendritic cells (MDC), and that it is efficiently presented by MDC to M1-specific CD8+ T lymphocytes.

Chapter 3

Hepatitis A

Hepatitis A	
ICD-10	B15.
ICD-9	070.1
DiseasesDB	5757
MedlinePlus	000278
eMedicine	med/991 ped/977
MeSH	D006506

Hepatitis A



Electron micrograph of hepatitis A virions.

Virus classification

Group:	Group IV ((+)ssRNA)
Family:	*Picornaviridae*
Genus:	*Hepatovirus*
Species:	*Hepatitis A virus*

Hepatitis A (formerly known as *infectious hepatitis*) is an acute infectious disease of the liver caused by the hepatitis A virus (HAV), which is transmitted person-to-person by ingestion of contaminated food or water or through direct contact with an infectious person. Tens of millions of individuals worldwide are estimated to become infected with HAV each year. The time between infection and the appearance of the symptoms (the incubation period) is between two and six weeks and the average incubation period is 28 days.

In developing countries, and in regions with poor hygiene standards, the incidence of infection with this virus is high and the illness is usually contracted in early childhood. As incomes rise and access to clean water increases, the incidence of HAV decreases. Hepatitis A infection causes no clinical signs and symptoms in over 90% of infected children and since the infection confers lifelong immunity, the disease is of no special significance to those infected early in life. In Europe, the United States and other industrialized countries, on the other hand, the infection is contracted primarily by susceptible young adults, most of whom are infected with the virus during trips to countries with a high incidence of the disease or through contact with infectious persons.

HAV infection produces a self-limited disease that does not result in chronic infection or chronic liver disease. However, 10%–15% of patients might experience a relapse of symptoms during the 6 months after acute illness. Acute liver failure from Hepatitis A is rare (overall case-fatality rate: 0.5%). The risk for symptomatic infection is directly related to age, with >80% of adults having symptoms compatible with acute viral hepatitis and the majority of children having either asymptomatic or unrecognized infection. Antibody produced in response to HAV infection persists for life and confers protection against reinfection. The disease can be prevented by vaccination, and hepatitis A vaccine has been proven effective in controlling outbreaks worldwide.

Signs and symptoms

Early symptoms of hepatitis A infection can be mistaken for influenza, but some sufferers, especially children, exhibit no symptoms at all. Symptoms typically appear 2 to 6 weeks, (the incubation period), after the initial infection.

Symptoms can return over the following 2–6 months and include:

- Fatigue
- Fever
- Abdominal pain
- Nausea

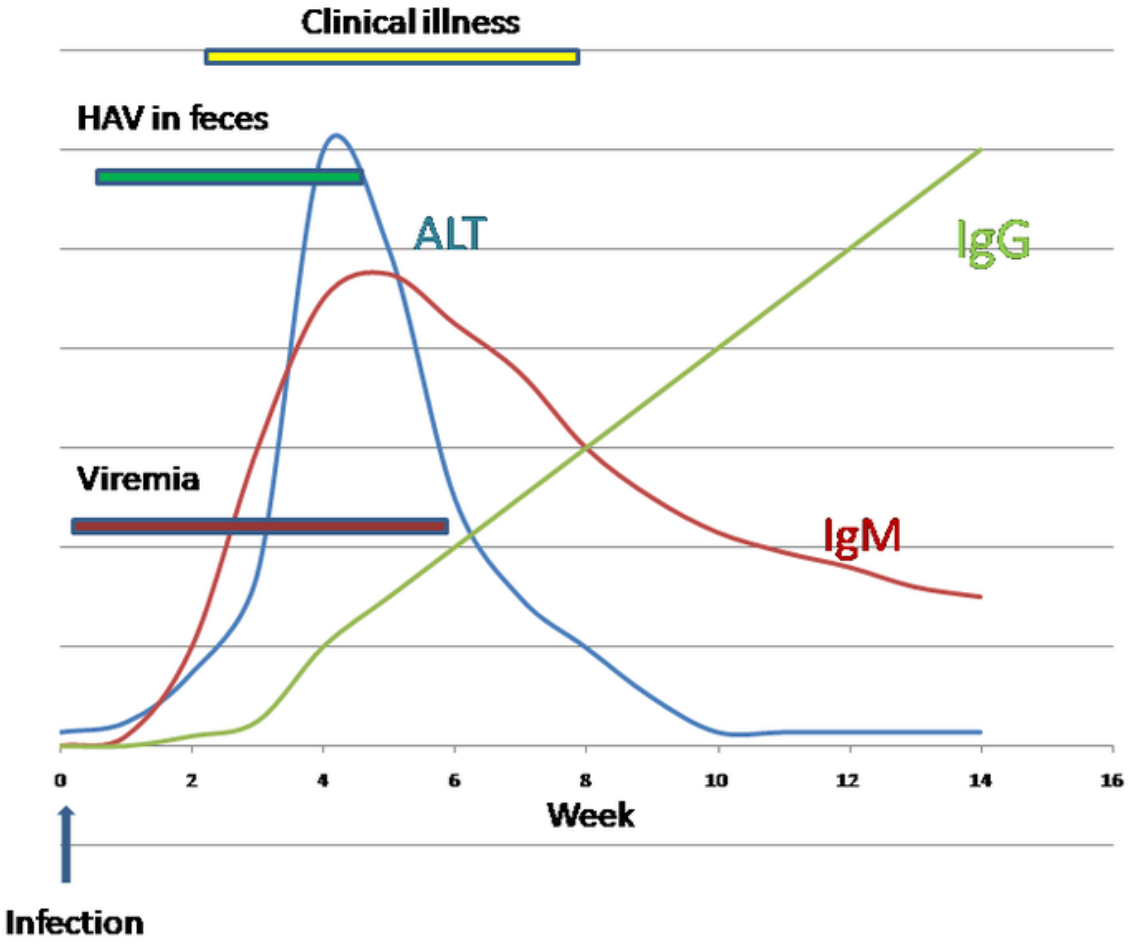
- Appetite loss
- Jaundice, a yellowing of the skin or whites of the eyes
- Bile is removed from blood stream and excreted in urine, giving it a dark amber colour
- Clay-coloured feces

Pathogenesis

Following ingestion, HAV enters the bloodstream through the epithelium of the oropharynx or intestine. The blood carries the virus to its target, the liver, where it multiplies within hepatocytes and Kupffer cells (liver macrophages). Virions are secreted into the bile and released in stool. HAV is excreted in large quantities approximately 11 days prior to appearance of symptoms or anti-HAV IgM antibodies in the blood. The incubation period is 15–50 days and mortality is less than 0.5%. Within the liver hepatocytes the RNA genome is released from the protein coat and is translated by the cell's own ribosomes. Unlike other members of the Picornaviruses this virus requires an intact eukaryote initiating factor 4G (eIF4G) for the initiation of translation. The requirement for this factor results in an inability to shut down host protein synthesis unlike other picornaviruses. The virus must then inefficiently compete for the cellular translational machinery which may explain its poor growth in cell culture. Presumably for this reason the virus has strategically adopted a naturally highly deoptimized codon usage with respect to that of its cellular host. Precisely how this strategy works is not quite clear yet.

There is no apparent virus-mediated cytotoxicity presumably because of the virus' own requirement for an intact eIF4G and liver pathology is likely immune-mediated.

Diagnosis



Serum IgG, IgM and ALT following Hepatitis A virus infection

Although HAV is excreted in the feces towards the end of the incubation period, specific diagnosis is made by the detection of HAV-specific IgM antibodies in the blood. IgM antibody is only present in the blood following an acute hepatitis A infection. It is detectable from one to two weeks after the initial infection and persists for up to 14 weeks. The presence of IgG antibody in the blood means that the acute stage of the illness is past and the person is immune to further infection. IgG antibody to HAV is also found in the blood following vaccination and tests for immunity to the virus are based on the detection of this antibody.

During the acute stage of the infection, the liver enzyme alanine transferase (ALT) is present in the blood at levels much higher than is normal. The enzyme comes from the liver cells that have been damaged by the virus.

Hepatitis A virus is present in the blood, (viremia), and feces of infected people up to two weeks before clinical illness develops.

Prevention

Hepatitis A can be prevented by vaccination, good hygiene and sanitation.

The vaccine protects against HAV in more than 95% of cases for longer than 20 years. It contains inactivated hepatitis A virus providing active immunity against a future infection. The vaccine was first phased in 1996 for children in high-risk areas, and in 1999 it was spread to areas with elevating levels of infection.

The vaccine is given by injection. An initial dose provides protection starting two to four weeks after vaccination; the second booster dose, given six to twelve months later, provides protection for over twenty years.

Treatment

There is no specific treatment for hepatitis A. Sufferers are advised to rest, avoid fatty foods and alcohol (these may be poorly tolerated for some additional months during the recovery phase and cause minor relapses), eat a well-balanced diet, and stay hydrated.

Pharmacotherapeutic goals are to reduce the morbidity and prevent complications involved in infection. The therapy is given by the agents like

- 1. Analgesics-to reduce the abdominal pain; usually Acetaminophen is given. Acetaminophen also acts as an antipyretic, to reduce the fever
- 2. Antiemetics-to suppress vomiting and nausea; usually Metoclopramide is given
- 3. Immune globulins usually BayGam 15-18% IG is given through intramuscular route

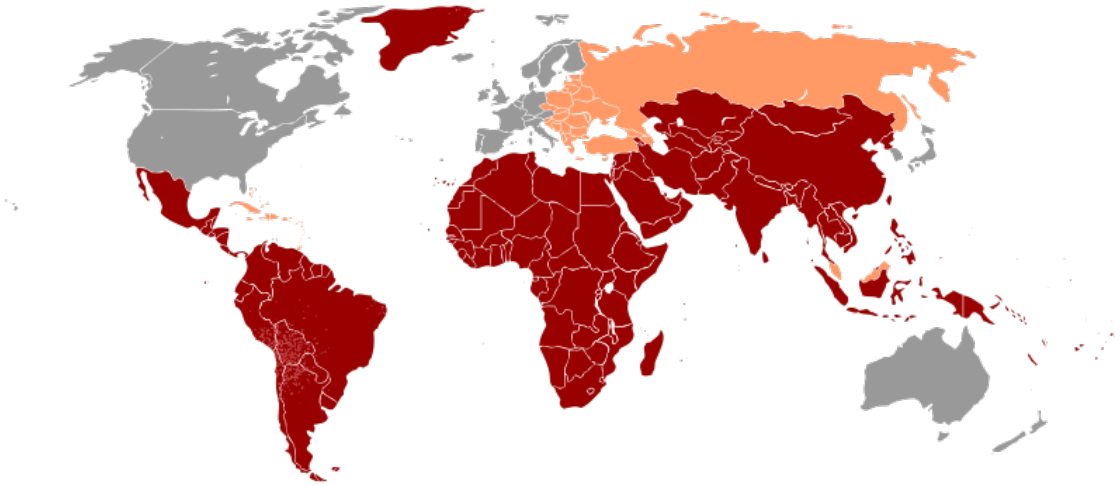
Prognosis

The United States Centers for Disease Control and Prevention (CDC) in 1991 reported a low mortality rate for hepatitis A of 4 deaths per 1000 cases for the general population but a higher rate of 17.5 per 1000 in those aged 50 and over. The risk of death from acute liver failure following HAV infection increases with age and when the person has underlying chronic liver disease.

Young children who are infected with hepatitis A typically have a milder form of the disease, usually lasting from 1–3 weeks, whereas adults tend to experience a much more severe form of the disease.

Epidemiology

Prevalence



Hepatitis A Distribution 2005

Antibodies to HAV (anti-HAV) in the blood are a marker of past or current infection. High-income regions (Western Europe, Australia, New Zealand, Canada, the United States, Japan, the Republic of Korea, and Singapore) have very low HAV endemicity levels and a high proportion of susceptible adults, low-income regions (sub-Saharan Africa and parts of South Asia) have high endemicity levels and almost no susceptible adolescents and adults, and most middle-income regions have a mix of intermediate and low endemicity levels. Anti-HAV prevalence suggest that middle-income regions in Asia, Latin America, Eastern Europe, and the Middle East currently have an intermediate or low level of endemicity. The countries in these regions may have an increasing burden of disease from hepatitis A.

There were 30,000 cases of Hepatitis A reported to the CDC in the U.S. in 1997. The agency estimates that there were as many as 270,000 cases each year from 1980 through 2000.

Transmission

The virus spreads by the fecal-oral route and infections often occur in conditions of poor sanitation and overcrowding. Hepatitis A can be transmitted by the parenteral route but very rarely by blood and blood products. Food-borne outbreaks are not uncommon, and ingestion of shellfish cultivated in polluted water is associated with a high risk of infection. Approximately 40% of all acute viral hepatitis is caused by HAV. Infected individuals are infectious prior to onset of symptoms, roughly 10 days following infection. The virus is resistant to detergent, acid (pH 1), solvents (e.g., ether, chloroform), drying, and temperatures up to 60^{o}C. It can survive for months in fresh and

salt water. Common-source (e.g., water, restaurant) outbreaks are typical. Infection is common in children in developing countries, reaching 100% incidence, but following infection there is life-long immunity. HAV can be inactivated by: chlorine treatment (drinking water), formalin (0.35%, 37^{o}C, 72 hours), peracetic acid (2%, 4 hours), beta-propiolactone (0.25%, 1 hour), and UV radiation (2 μW/cm^{2}/min).

Cases

The most widespread hepatitis A outbreak in the United States afflicted at least 640 people (killing four) in north-eastern Ohio and south-western Pennsylvania in late 2003. The outbreak was blamed on tainted green onions at a restaurant in Monaca, Pennsylvania. In 1988, 300,000 people in Shanghai, China were infected with HAV after eating clams from a contaminated river.

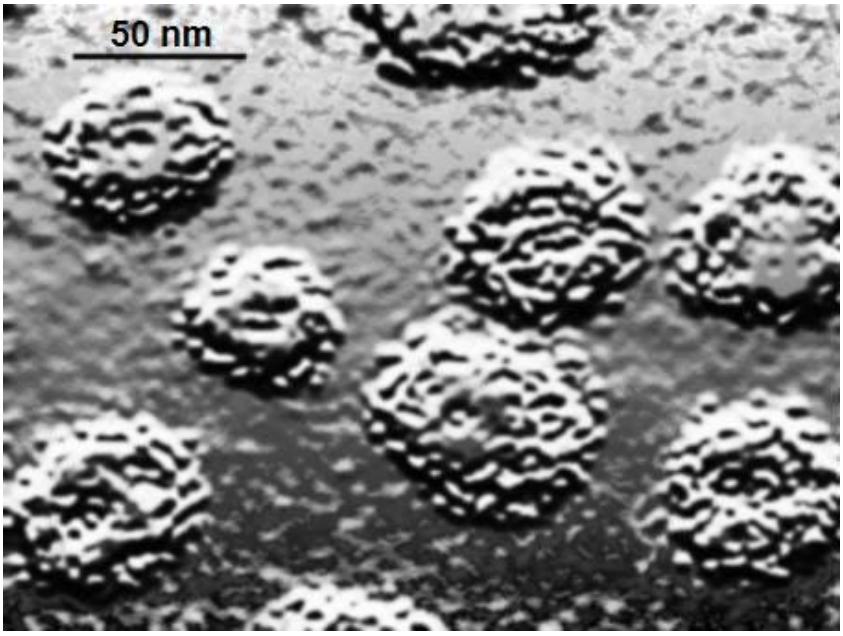
Virology

The Hepatitis virus (HAV) is a Picornavirus; it is non-enveloped and contains a single-stranded RNA packaged in a protein shell. There is only one serotype of the virus, but multiple genotypes exist. Codon use within the genome is biased and unusually distinct from its host. It also has a poor internal ribosome entry site In the region that codes for the HAV capsid there are highly conserved clusters of rare codons that restrict antigenic variability.

Chapter 4

Papillomaviridae

Papillomavirus



EM of papillomavirus

Virus classification

Group: Group I (dsDNA)

Family: ***Papillomaviridae***

Genera

Alphapapillomavirus
Betapapillomavirus
Gammapapillomavirus
Deltapapillomavirus
Epsilonpapillomavirus
Etapapillomavirus
Iotapapillomavirus
Kappapapillomavirus
Lambdapapillomavirus
Mupapillomavirus
Nupapillomavirus

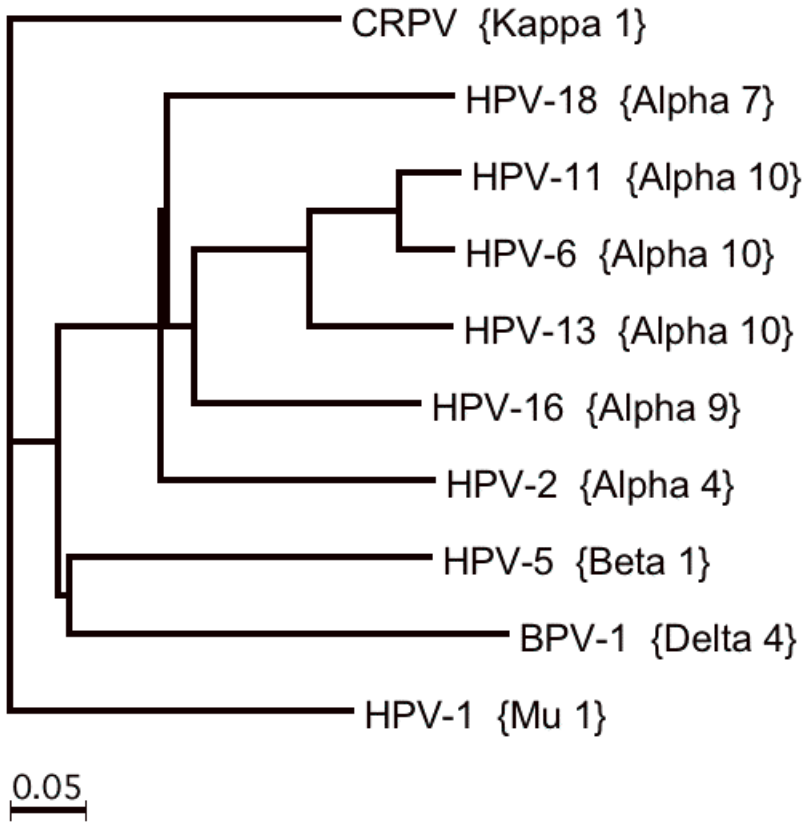
Omikronpapillomavirus
Pipapapillomavirus
Thetapapillomavirus
Xipapillomavirus
Zetapapillomavirus

Papillomaviridae is an ancient and diverse taxonomic family of non-enveloped DNA viruses, collectively known as *papillomaviruses*. Several hundred species of papillomaviruses, traditionally referred to as "types", have been identified infecting all carefully inspected birds and mammals, but also a small number of reptiles, in particular turtles and tortoises. Infection by most papillomavirus types, depending on the type, is either asymptomatic or causes small benign tumors, known as papillomas or warts. Papillomas caused by some types, however, such as human papillomaviruses 16 and 18, carry a risk of becoming cancerous.

Papillomaviruses are highly host- and tissue-tropic, and are rarely transmitted between species. Papillomaviruses replicate exclusively in the basal layer of the body surface tissues. All known papillomavirus types infect a particular body surface, typically the skin or mucosal epithelium of the genitals, anus, mouth, or airways. For example, human papillomavirus (HPV) type 1 tends to infect the soles of the feet, and HPV type 2 the palms of the hands, where they may cause warts.

Papillomaviruses were first identified in the early 20th century, when it was shown that skin warts, or papillomas, could be transmitted between individuals by a filterable infectious agent. In 1935 Francis Peyton Rous, who had previously demonstrated the existence of a cancer-causing sarcoma virus in chickens, went on to show that a papillomavirus could cause skin cancer in infected rabbits. This was the first demonstration that a virus could cause cancer in mammals.

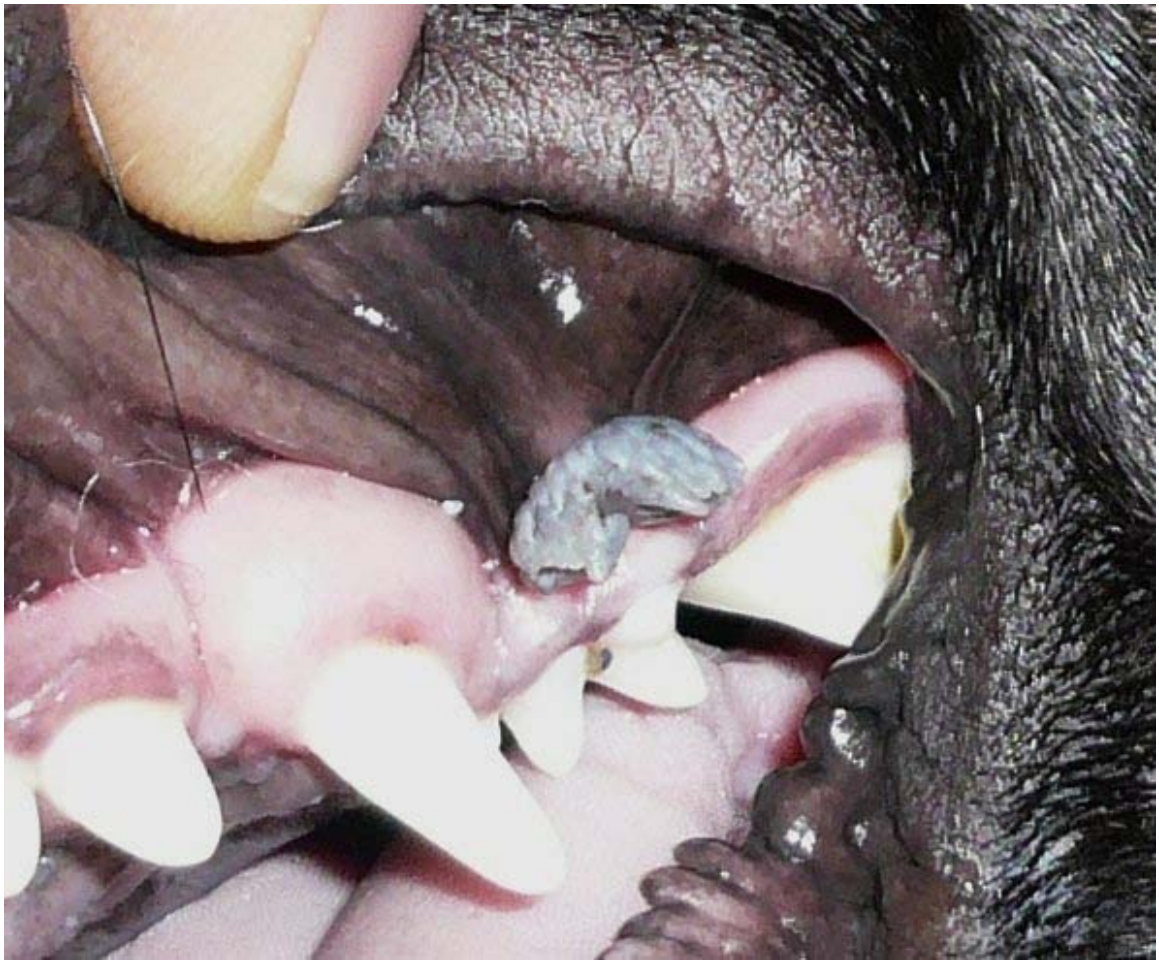
Taxonomy of papillomaviruses



Selected papillomavirus types

Papillomaviruses (PVs) have similar genomic organizations, and any pair of PVs contains at least five homologous genes, although the nucleotide sequence may diverge by more than 50%. Phylogenetic algorithms that permit the comparison of homologies led to phylogenetic trees that have a similar topology, independent of the gene analyzed. Phylogenetic studies strongly suggest that PVs normally evolve together with their mammalian and bird host species, do not change host species, do not recombine, and have maintained their basic genomic organization for a period exceeding 100 million years. These sequence comparisons have laid the foundation for a PV taxonomy, which is now officially recognized by the International Committee on Taxonomy of Viruses. All PVs form the family *Papillomaviridae*, which is distinct from the *Polyomaviridae* thus eliminating the term *Papovaviridae*. Major branches of the phylogenetic tree of PVs are considered genera, which are identified by Greek letters. Minor branches are considered species and unite PV types that are genomically distinct without exhibiting known biological differences. This new taxonomic system does not affect the traditional identification and characterization of PV "types" and their independent isolates with minor genomic differences, referred to as "subtypes" and "variants", all of which are taxa below the level of "species".

Animal papillomaviruses



Viral papilloma in a dog

Individual papillomavirus types tend to be highly adapted to replication in a single animal species. In one study, researchers swabbed the forehead skin of a variety of zoo animals and used PCR to amplify any papillomavirus DNA that might be present. Although a wide variety of papillomavirus sequences were identified in the study, the authors found little evidence for inter-species transmission. Interestingly, one zookeeper was found to be transiently positive for a chimpanzee-specific papillomavirus sequence. However, the authors note that the chimpanzee-specific papillomavirus sequence could have been the result of surface contamination of the zookeeper's skin, as opposed to productive infection.

Cottontail rabbit papillomavirus (CRPV) can cause protuberant warts in its native host, the North American rabbit genus *Sylvilagus*. These horn-like warts may be the original basis for the urban legends of the American antlered rabbit the Jackalope and European *Wolpertinger*. European domestic rabbits (genus *Oryctolagus*) can be transiently infected with CRPV in a laboratory setting. However, since European domestic rabbits do not

produce infectious progeny virus, they are considered an incidental or "dead-end" host for CRPV.

Inter-species transmission has also been documented for bovine papillomavirus (BPV) type 1. In its natural host (cattle), BPV-1 induces large fibrous skin warts. BPV-1 infection of horses, which are an incidental host for the virus, can lead to the development of benign tumors known as sarcoids. The agricultural significance of BPV-1 spurred a successful effort to develop a vaccine against the virus.

A few reports have identified papillomaviruses in smaller rodents, such as Syrian hamsters, the African multimammate rat and the European harvest mouse. However, there are no papillomaviruses known to be capable of infecting laboratory mice. The lack of a tractable mouse model for papillomavirus infection has been a major limitation for laboratory investigation of papillomaviruses.

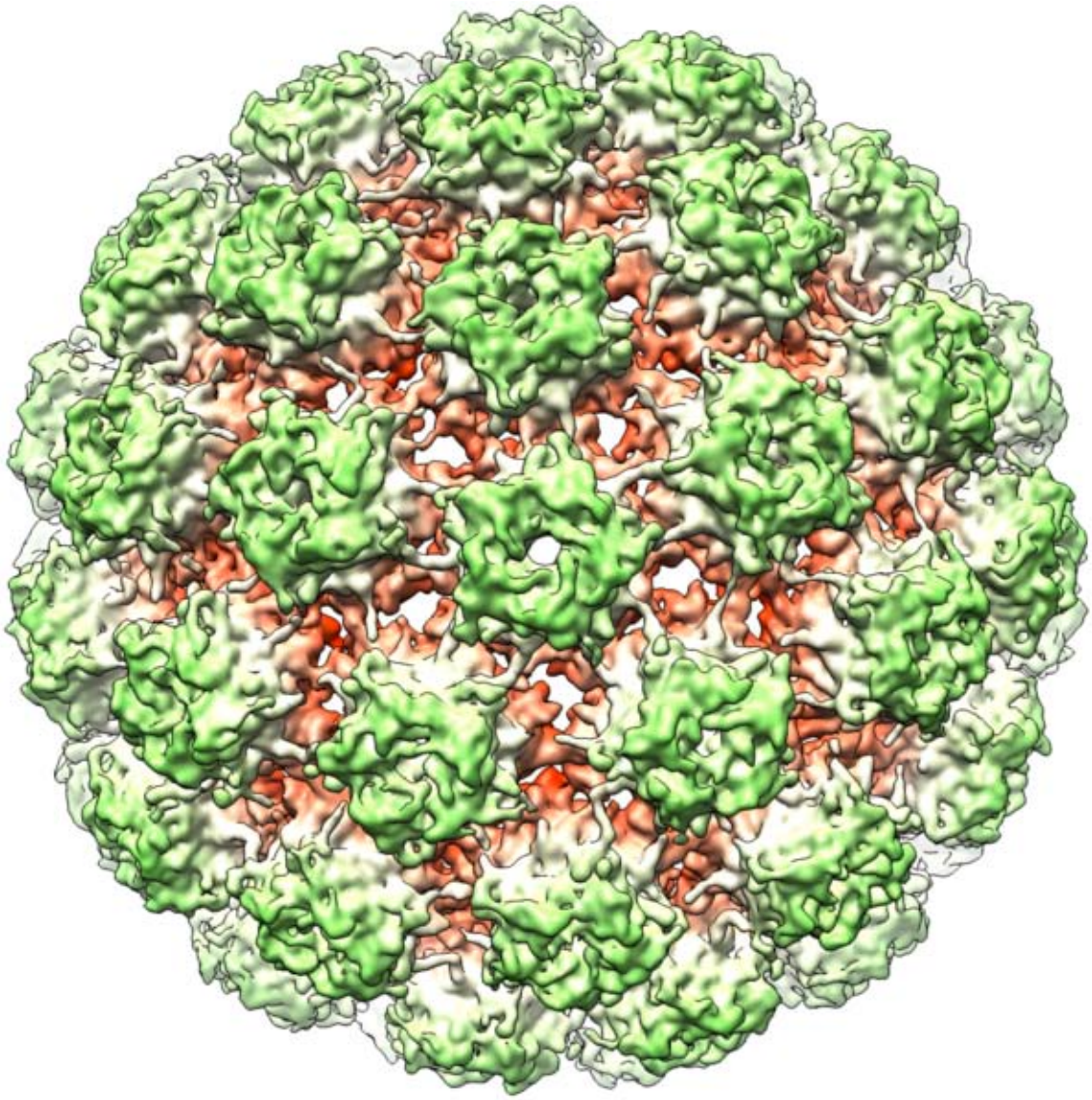
Evolution

The evolution of papillomaviruses is slow compared to many other virus types. This is probably because the papillomavirus genome is composed of genetically stable double-stranded DNA that is replicated with high fidelity by the host cell's DNA replication machinery.

It is believed that papillomaviruses generally co-evolve with a particular species of host animal over many years. In a particularly speedy example, HPV-16 has evolved slightly as human populations have expanded across the globe and now varies in different geographic regions in a way that probably reflects the history of human migration.

Other HPV types, such as HPV-13, vary relatively little in different human populations. In fact, the sequence of HPV-13 closely resembles a papillomavirus of bonobos (also known as pygmy chimpanzees). It is not clear whether this similarity is due to recent transmission between species or because HPV-13 has simply changed very little in the six or so million years since humans and bonobos diverged.

Structure



Papillomavirus capsid from Bovine

Papillomaviruses are non-enveloped, meaning that the outer shell or capsid of the virus is not covered by a lipid membrane. A single viral protein, known as L1, is necessary and sufficient for formation of a 60 nanometer capsid composed of 72 star-shaped capsomers (see figure). Like most non-enveloped viruses, the capsid is geometrically regular and presents icosahedral symmetry. Self-assembled virus-like particles composed of L1 are the basis of a successful group of prophylactic HPV vaccines designed to elicit virus-neutralizing antibodies that protect against initial HPV infection.

The papillomavirus genome is a double-stranded circular DNA molecule ~8,000 base pairs in length. It is packaged within the L1 shell along with cellular histone proteins, which serve to wrap and condense DNA.

The papillomavirus capsid also contains a viral protein known as L2, which is less abundant. Although not clear how L2 is arranged within the virion, it is known to perform several important functions, including facilitating the packaging of the viral genome into nascent virions as well as the infectious entry of the virus into new host cells. L2 is of interest as a possible target for more broadly protective HPV vaccines.

Tissue specificity

Papillomaviruses replicate exclusively in keratinocytes. Keratinocytes form the outermost layers of the skin, as well as some mucosal surfaces, such as the inside of the cheek or the walls of the vagina. These surface tissues, which are known as stratified squamous epithelia, are composed of stacked layers of flattened cells. The cell layers are formed through a process known as cellular differentiation, in which keratinocytes gradually become specialized, eventually forming a hard, crosslinked surface that prevents moisture loss and acts as a barrier against pathogens. Less-differentiated keratinocyte stem cells, replenished on the surface layer, are thought to be the initial target of productive papillomavirus infections. Subsequent steps in the viral life cycle are strictly dependent on the process of keratinocyte differentiation. As a result, papillomaviruses can only replicate in body surface tissues.

Life cycle

Infectious entry

Papillomaviruses gain access to keratinocyte stem cells through small wounds, known as microtraumas, in the skin or mucosal surface. Interactions between L1 and sulfated sugars on the cell surface promote initial attachment of the virus. The virus is then able to get inside from the cell surface via interaction with a specific receptor, likely via the alpha-6 beta-4 integrin, and transported to membrane-enclosed vesicles called endosomes. The capsid protein L2 disrupts the membrane of the endosome, allowing the viral genome to escape and traffic, along with L2, to the cell nucleus.

Viral Persistence

After successful infection of a keratinocyte, the virus expresses E1 and E2 proteins, which are for replicating and maintaining the viral DNA as a circular episome. The viral oncogenes E6 and E7 promote cell growth by inactivating the tumor suppressor proteins p53 and pRb. Keratinocyte stem cells in the epithelial basement layer can maintain papillomavirus genomes for decades.

Production of progeny virus

The expression of the viral late genes, L1 and L2, is exclusively restricted to differentiating keratinocytes in the outermost layers of the skin or mucosal surface. The increased expression of L1 and L2 is typically correlated with a dramatic increase in the number of copies of the viral genome. Since the outer layers of stratified squamous epithelia are subject to relatively limited surveillance by cells of the immune system, it is thought that this restriction of viral late gene expression represents a form of immune evasion.

New infectious progeny virus are assembled in the cell nucleus. Papillomaviruses have evolved a mechanism for releasing virions into the environment. Other kinds of non-enveloped animal viruses utilize an active lytic process to kill the host cell, allowing release of progeny virus particles. Often this lytic process is associated with inflammation, which might trigger immune attack against the virus. Papillomaviruses exploit desquamation as a stealthy, non-inflammatory release mechanism.

Cancer

Although some papillomavirus types can cause cancer in the epithelial tissues they inhabit, cancer is not a typical outcome of infection. The development of papillomavirus-induced cancers typically occurs over the course of many years.

Laboratory study

The fact that the papillomavirus life cycle strictly requires keratinocyte differentiation has posed a substantial barrier to the study of papillomaviruses in the laboratory, since it has precluded the use of conventional cell lines to grow the viruses. Because infectious BPV-1 virions can be extracted from the large warts the virus induces on cattle, it has been a workhorse model papillomavirus type for many years. CRPV, rabbit oral papillomavirus (ROPV) and canine oral papillomavirus (COPV) have also been used extensively for laboratory studies.

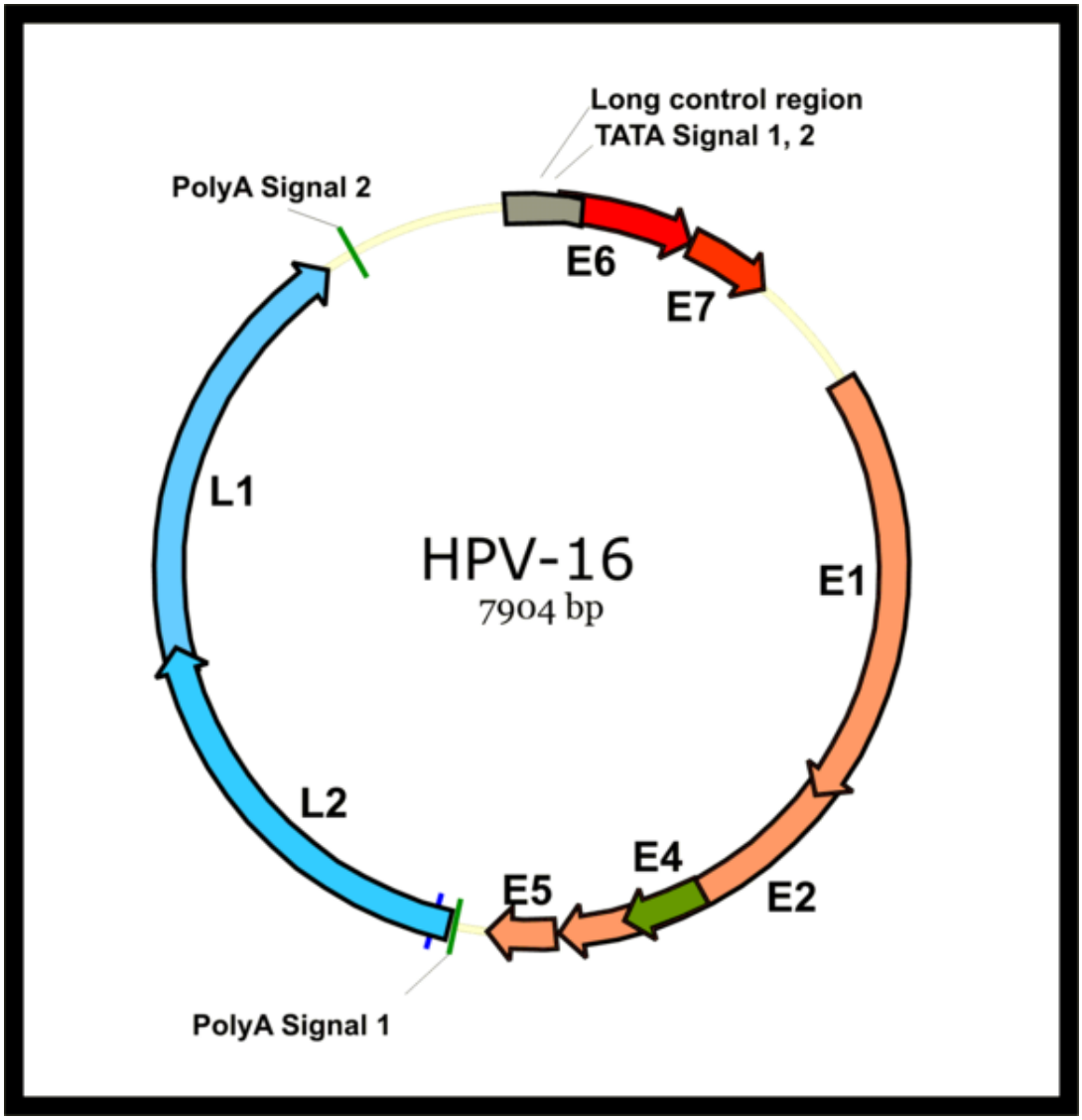
Some sexually transmitted HPV types have been propagated using a mouse “xenograft” system, in which HPV-infected human cells are implanted into immunodeficient mice. More recently, some groups have succeeded in isolating infectious HPV-16 from human cervical lesions. However, isolation of infectious virions using this technique is arduous and the yield of infectious virus is very low.

The differentiation of keratinocytes can be mimicked *in vitro* by exposing cultured keratinocytes to an air/liquid interface. The adaptation of such “raft culture” systems to the study of papillomaviruses was a significant breakthrough for *in vitro* study of the viral life cycle. However, raft culture systems are relatively cumbersome and the yield of infectious HPVs can be low.

The development of a yeast-based system that allows stable episomal HPV replication provides a convenient, rapid and inexpensive means to study several aspects of the HPV lifecycle (Angeletti 2002). For example, E2-dependent transcription, genome amplification and efficient encapsidation of full-length HPV DNAs can be easily recreated in yeast (Angeletti 2005).

Recently, transient high-yield methods for producing HPV pseudoviruses carrying reporter genes has been developed. Although pseudoviruses are not suitable for studying certain aspects of the viral life cycle, initial studies suggest that their structure and initial infectious entry into cells is probably similar in many ways to authentic papillomaviruses.

Genetic organization



Genome organization of Human papillomavirus type 16

The papillomavirus genome is divided into an early region (E), encoding various genes that are expressed immediately after initial infection of a host cell, and a late region (L) encoding the capsid genes L1 and L2. All the genes are encoded on one DNA strand (see figure). This represents a dramatic difference between papillomaviruses and polyomaviruses, since the latter virus type expresses its early and late genes by bi-directional transcription of both DNA strands. This difference was a major factor in establishment of the consensus that papillomaviruses and polyomaviruses probably never shared a common ancestor, despite the striking similarities in the structures of their virions.

Technical discussion of papillomavirus gene functions

Genes within the papillomavirus genome are usually identified after similarity with other previously identified genes. However, some spurious open reading frames might have been mistaken as genes simply after their position in the genome, and might not be true genes. This applies specially to certain E3, E4, E5 and E8 open reading frames.

E1

Encodes a protein that binds to the viral origin of replication in the long control region of the viral genome. E1 uses ATP to exert a helicase activity that forces apart the DNA strands, thus preparing the viral genome for replication by cellular DNA replication factors.

E2

The E2 protein serves as a master transcriptional regulator for viral promoters located primarily in the long control region. The protein has a transactivation domain linked by a relatively unstructured hinge region to a well-characterized DNA binding domain. E2 facilitates the binding of E1 to the viral origin of replication. E2 also utilizes a cellular protein known as Bromodomain-4 (Brd4) to tether the viral genome to cellular chromosomes. This tethering to the cell's nuclear matrix ensures faithful distribution of viral genomes to each daughter cell after cell division. It is thought that E2 serves as a negative regulator of expression for the oncogenes E6 and E7 in latently HPV-infected basal layer keratinocytes. Genetic changes, such as integration of the viral DNA into a host cell chromosome, that inactivate E2 expression tend to increase the expression of the E6 and E7 oncogenes, resulting in cellular transformation and possibly further genetic destabilization.

E3

This small putative gene exists only in a few papillomavirus types. The gene is not known to be expressed as a protein and does not appear to serve any function.

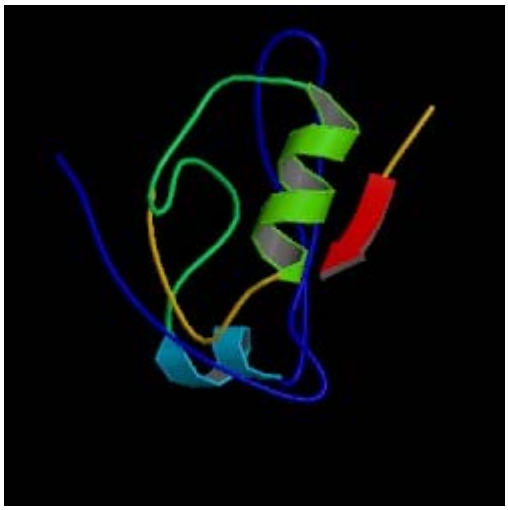
E4

Although E4 proteins are expressed at low levels during the early phase of viral infection, expression of E4 increases dramatically during the late phase of infection. In other words, its “E” appellation may be something of a misnomer. In the case of HPV-1, E4 can account for up to 30% of the total protein at the surface of a wart. The E4 protein of many papillomavirus types is thought to facilitate virion release into the environment by disrupting intermediate filaments of the keratinocyte cytoskeleton. Viral mutants incapable of expressing E4 do not support high-level replication of the viral DNA, but it is not yet clear how E4 facilitates DNA replication. E4 has also been shown to participate in arresting cells in the G2 phase of the cell cycle.

E5

The E5 are small, very hydrophobic proteins that destabilise the function of many membrane proteins in the infected cell. The E5 protein of some animal papillomavirus types (mainly bovine papillomavirus type 1) functions as an oncogene primarily by activating the cell growth-promoting signaling of platelet-derived growth factor receptors. The E5 proteins of human papillomaviruses associated to cancer, however, seem to activate the signal cascade initiated by epidermal growth factor upon ligand binding. HPV16 E5 and HPV2 E5 have also been shown to down-regulate the surface expression of major histocompatibility complex class I proteins, which may prevent the infected cell from being eliminated by killer T cells.

E6



Structure of Sap97 PDZ3 bound to the C-terminal peptide of HPV18 E6 (PDB 2I0I)

E6 is a 151 amino-acid peptide that incorporates a type 1 motif with a consensus sequence –(T/S)-(X)-(V/I)-COOH. It also has two zinc finger motifs.

E6 is of particular interest because it appears to have multiple roles in the cell and to interact with many other proteins. Its major role, however, is to mediate the degradation of p53, a major tumor suppressor protein, reducing the cell's ability to respond to DNA damage.

E6 has also been shown to target other cellular proteins, thereby altering several metabolic pathways. One such target is NFX1-91, which normally represses production of telomerase, a protein that allows cells to divide an unlimited number of times. When NFX1-91 is degraded by E6, telomerase levels increase, inactivating a major mechanism keeping cell growth in check. Additionally, E6 can act as a transcriptional cofactor—specifically, a transcription activator—when interacting with the cellular transcription factor, E2F1/DP1.

E6 can also bind to PDZ-domains, short sequences which are often found in signaling proteins. E6's structural motif allows for interaction with PDZ domains on DLG (discs large) and hDLG (Drosophila large) tumor suppressor genes. Binding at these locations causes transformation of the DLG protein and disruption of its suppressor function. E6 proteins also interact with the MAGUK (membrane-associated guanylate kinase family) proteins. These proteins, including MAGI-1, MAGI-2, and MAGI-3 are usually structural proteins, and can help with signaling. More significantly, they are believed to be involved with DLG's suppression activity. When E6 complexes with the PDZ domains on the MAGI proteins, it distorts their shape and thereby impedes their function. Overall, the E6 protein serves to impede normal protein activity in such a way as to allow a cell to grow and multiply at the increased rate characteristic of cancer.

Since the expression of E6 is strictly required for maintenance of a malignant phenotype in HPV-induced cancers, it is an appealing target of therapeutic HPV vaccines designed to eradicate established cervical cancer tumors.

E7

In most papillomavirus types, the primary function of the E7 protein is to inactivate members of the pRb family of tumor suppressor proteins. Together with E6, E7 serves to prevent cell death (apoptosis) and promote cell cycle progression, thus priming the cell for replication of the viral DNA. E7 also participates in immortalization of infected cells by activating cellular telomerase. Like E6, E7 is the subject of intense research interest and is believed to exert a wide variety of other effects on infected cells. As with E6, the ongoing expression of E7 is required for survival of cancer cell lines, such as HeLa, that are derived from HPV-induced tumors.

E8

Only a few papillomavirus types encode a short protein from the E8 gene. In the case of BPV-4 (papillomavirus genus *Xi*), the E8 open reading frame may substitute for the E6 open reading frame, which is absent in this papillomavirus genus. These E8 genes are

chemically and functionally similar to the E5 genes from some human papillomaviruses, and are also called E5/E8.

L1

L1 spontaneously self-assembles into pentameric capsomers. Purified capsomers can go on to form capsids, which are stabilized by disulfide bonds between neighboring L1 molecules. L1 capsids assembled *in vitro* are the basis of prophylactic vaccines against several HPV types. Compared to other papillomavirus genes, the amino acid sequences of most portions of L1 are well-conserved between types. However, the surface loops of L1 can differ substantially, even for different members of a particular papillomavirus species. This probably reflects a mechanism for evasion of neutralizing antibody responses elicited by previous papillomavirus infections.

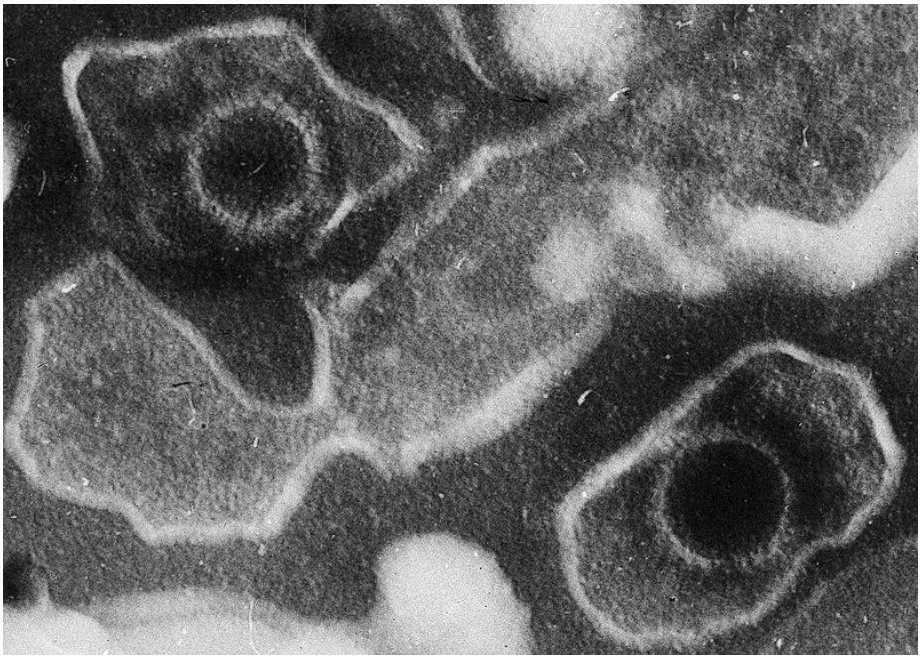
L2

L2 exists in an oxidized state within the papillomavirus virion, with the two conserved cysteine residues forming an intramolecular disulfide bond. In addition to cooperating with L1 to package the viral DNA into the virion, L2 has been shown to interact with a number of cellular proteins during the infectious entry process. After the initial binding of the virion to the cell, L2 must be cleaved by the cellular protease furin. The virion is internalized, probably through a clathrin-mediated process, into an endosome, where acidic conditions are thought to lead to exposure of membrane-destabilizing portions of L2. The cellular proteins beta-actin and syntaxin-18 may also participate in L2-mediated entry events. After endosome escape, L2 and the viral genome are imported into the cell nucleus where they traffic to a sub-nuclear domain known as an ND-10 body that is rich in transcription factors. Small portions of L2 are well-conserved between different papillomavirus types, and experimental vaccines targeting these conserved domains may offer protection against a broad range of HPV types.

Chapter 5

Epstein-Barr Virus

Epstein-Barr



Two Epstein-Barr virions

Virus classification	
Group:	Group I (dsDNA)
Family:	*Herpesviridae*
Subfamily:	*Gammaherpesvirinae*
Genus:	*Lymphocryptovirus*
Species:	***Human herpesvirus 4*** (HHV-4)

The **Epstein-Barr virus** (**EBV**), also called **human herpesvirus 4** (**HHV-4**), is a virus of the herpes family, which includes *herpes simplex virus* 1 and 2, and is one of the most common viruses in humans. It is best known as the cause of infectious mononucleosis. It is also associated with particular forms of cancer, particularly Hodgkin's lymphoma, Burkitt's lymphoma, nasopharyngeal carcinoma, and central nervous system lymphomas associated with HIV. Finally, there is evidence that infection with the virus is associated

with a higher risk of certain autoimmune diseases, especially dermatomyositis, systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome, and multiple sclerosis.

Most people become infected with EBV and gain adaptive immunity. In the United States, about half of all five-year-olds and 90–95% of adults have evidence of previous infection. Infants become susceptible to EBV as soon as maternal antibody protection disappears. Many children become infected with EBV, and these infections usually cause no symptoms or are indistinguishable from the other mild, brief illnesses of childhood. In the United States and in other developed countries, many people are not infected with EBV in their childhood years. When infection with EBV occurs during adolescence or teenage years, it causes infectious mononucleosis 35% to 69% of the time.

History

Epstein-Barr virus is named after M. Anthony Epstein and Yvonne Barr, who discovered and documented the virus. In 1961, M. Anthony Epstein, a pathologist and expert electron microscopist, attended a lecture on "The Commonest Children's Cancer in Tropical Africa — A Hitherto Unrecognised Syndrome." This lecture, by Denis Parsons Burkitt, a surgeon practicing in Uganda, was the description of the "endemic variant" (pediatric form) of the disease that bears his name. In 1963, a specimen was sent from Uganda to Middlesex Hospital to be cultured. Virus particles were identified in the cultured cells, and the results were published in The Lancet in 1964 by Epstein, Bert Achong, and Barr. Cell lines were sent to Werner and Gertrude Henle at the Children's Hospital of Philadelphia who developed serological markers. In 1967, a technician in their laboratory developed mononucleosis and they were able to compare a stored serum sample, showing that antibodies to the virus developed.

Virology

The virus can execute many distinct programs of gene expression which can be broadly categorized as being *lytic* cycle or *latent* cycle.

- The lytic cycle or productive infection results in staged expression of several viral proteins with the ultimate objective of producing infectious virions. Formally, this phase of infection does not inevitably lead to lysis of the host cell as EBV virions are produced by budding from the infected cell. Lytic proteins include gp350 and gp110.
- The latent cycle (lysogenic) programs are those that do not result in production of virions. A very limited, distinct set of viral proteins are produced during latent cycle infection. These include Epstein-Barr nuclear antigen (EBNA)-1, EBNA-2, EBNA-3A, EBNA-3B, EBNA-3C, EBNA-leader protein (EBNA-LP) and latent membrane proteins (LMP)-1, LMP-2A and LMP-2B and the Epstein-Barr encoded RNAs (EBERs). In addition, EBV codes for at least twenty microRNAs which are expressed in latently infected cells and at least one snoRNA expressed during lytic cycle.

Programs

From studies of EBV gene expression in cultured Burkitt's lymphoma cell lines, at least three programs exist:

1. EBER1&2 LMP2A EBNA1 (Latency I)
2. EBER1&2 LMP2A LMP2B EBNA1 LMP1 (Latency II)
3. EBER1&2 LMP2A LMP2B EBNA1 LMP1 EBNA2,3,4,5,6 (Latency III)

It is also postulated that a program exists in which all viral protein expression is shut off(latency 0).

Latent cycle

Epstein-Barr virus and its sister virus KSHV can be maintained and manipulated in the laboratory in continual latency. While many viruses are assumed to have this property during infection of their natural host, they do not have an easily managed system for studying this part of the viral lifecycle. Further, Walter Henle and Gertrude Henle, together with Harald zur Hausen, who later discovered the papillomaviruses that causes cervical cancer, discovered that EBV can directly immortalize B cells after infection, mimicking some forms of EBV-related neoplasia.

On infecting the B-lymphocyte by binding to the complement receptor, the linear genome circularizes and the virus subsequently persists within the cell as an episome.

In primary infection, EBV replicates in oro-pharyngeal epithelial cells and establishes Latency III, II, and I infections in B-lymphocytes. EBV latent infection of B-lymphocytes is necessary for virus persistence, subsequent replication in epithelial cells, and release of infectious virus into saliva. EBV Latency III and II infections of B-lymphocytes, Latency II infection of oral epithelial cells, and Latency II infection of NK- or T-cell can result in malignancies, marked by uniform EBV genome presence and gene expression.

Transformation

When EBV infects B-lymphocytes in vitro, lymphoblastoid cell lines eventually emerge that are capable of indefinite growth. The growth transformation of these cell lines is the consequence of viral protein expression.

EBNA-2, EBNA-3C and LMP-1 are essential for transformation while EBNA-LP and the EBERs are not. The EBNA-1 protein is essential for maintenance of the virus genome.

It is postulated that following natural infection with EBV, the virus executes some or all of its repertoire of gene expression programs to establish a persistent infection. Given the initial absence of host immunity, the lytic cycle produces large amounts of virus to infect other (presumably) B-lymphocytes within the host.

The latent programs reprogram and subvert infected B-lymphocytes to proliferate and bring infected cells to the sites at which the virus presumably persists. Eventually, when host immunity develops, the virus persists by turning off most (or possibly all) of its genes, only occasionally reactivating to produce fresh virions. A balance is eventually struck between occasional viral reactivation and host immune surveillance removing cells that activate viral gene expression.

The site of persistence of EBV may be bone marrow. EBV-positive patients who have had their own bone marrow replaced with bone marrow from an EBV-negative donor are found to be EBV-negative after transplantation.

Latent antigens

All EBV nuclear proteins are produced by alternative splicing of a transcript starting at either the Cp or Wp promoters at the left end of the genome (in the conventional nomenclature). The genes are ordered EBNA-LP/EBNA-2/EBNA-3A/EBNA-3B/EBNA-3C/EBNA-1 within the genome.

The initiation codon of the **EBNA-LP** coding region is created by an alternate splice of the nuclear protein transcript. In the absence of this initiation codon, EBNA-2/EBNA-3A/EBNA-3B/EBNA-3C/EBNA-1 will be expressed depending on which of these genes is alternatively spliced into the transcript.

Viral entry

EBV can infect a number of different cell types, including B cells and epithelial cells, and under certain cases, it may infect T cells, natural killer cells, and smooth muscle cells. Infecting both the B cells and the epithelial cells is part of the viral normal cycle to persist. However, the entry mechanism and the proteins involved in entry for these two cells are different.

To infect B cells, the gp350 viral protein binds to the cellular receptor complement receptor 2 (CR2), and triggers endocytosis. In addition, gp42 binds to MHC class II molecule. Through these interactions, the fusion machinery, composed of gHgL and gB, is triggered and the viral membrane fuses with the endosomal membrane to release viral genetic materials.

To infect epithelial cells, gp350 also binds to CR2; however, endocytosis is not triggered. Then, gHgL interacts with a gHgL receptor (possibly integrins $\alpha v\beta 6$ or $\alpha v\beta 8$) and the fusion machinery gHgL and gB is triggered to allow fusion on cell membrane. Fusion with epithelial cells is actually impeded by gp42.

Tropism

The three-part complexes of gHgLgp42 mediate B cell membrane fusion; while the two-part complexes of gHgL mediate epithelial cell membrane fusion. EBV that are made in

the B cells have low numbers of the gHgLgp42 complexes as the three-part complexes interact with HLA class II in the endoplasmic reticulum and are degraded. In contrast, EBV from epithelial cells are rich in the three-part complexes because these cells do not have MHC class II. As a result, EBV made from B cells are more infectious to epithelial cells, and EBV made from epithelial cells are more infectious to B cells.

Protein/genes

Protein/gene/antigen	Stage	Description
EBNA-1	latent+lytic	EBNA-1 protein binds to a replication origin (oriP) within the viral genome and mediates replication and partitioning of the episome during division of the host cell. It is the only viral protein expressed during group I latency.
EBNA-2	latent+lytic	EBNA-2 is the main viral transactivator.
EBNA-3	latent+lytic	These genes also bind the host RBP-Jκ protein.
LMP-1	latent	LMP-1 is a six-span transmembrane protein that is also essential for EBV-mediated growth transformation.
LMP-2	latent	LMP-2A/LMP-2B are transmembrane proteins that act to block tyrosine kinase signaling.
EBER	latent	EBER-1/EBER-2 are small nuclear RNAs, which bind to certain nucleoprotein particles, enabling binding to PKR (dsRNA dependent serin/threonin protein kinase) thus inhibiting its function. EBER-particles also induce the production of IL-10 which enhances growth and inhibits cytotoxic T-cells.
miRNAs	latent	EBV microRNAs are encoded by two transcripts, one set in the BART gene and one set near the BHRF1 cluster. The three BHRF1 miRNAS are expressed during type III latency while the large cluster of BART miRNAs (up to 20 miRNAs) are expressed during type II latency. The functions of these miRNAs are currently unknown.
EBV-EA	lytic	early antigen
EBV-MA	lytic	membrane antigen
EBV-VCA	lytic	viral capsid antigen

EBV-AN	lytic	alkaline nuclease

Surface receptors

The Epstein-Barr Virus surface glycoprotein H (gH) is essential for penetration of B cells but also plays a role in attachment of virus to epithelial cells.

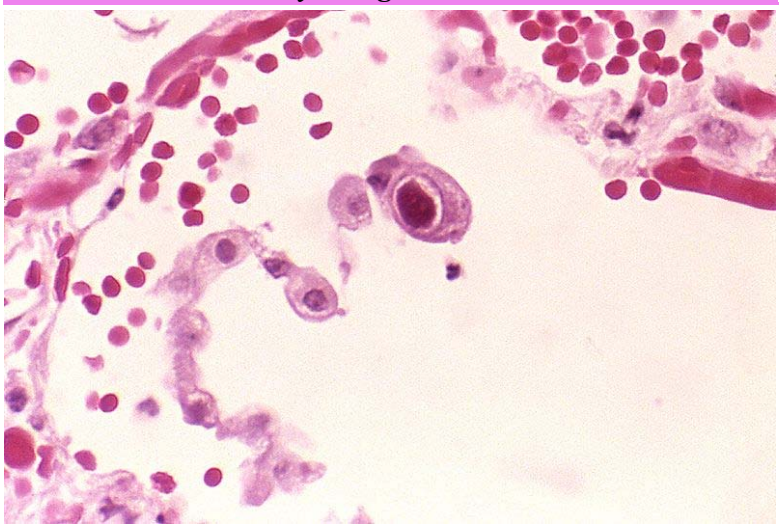
In laboratory and animal trials in 2000, it was shown that both antagonism of RA-mediated growth inhibition and promotion of LCL proliferation were efficiently reversed by the glucocorticoid receptor (GR) antagonist RU486.

Chapter 6

Cytomegalovirus

Cytomegalovirus	
ICD-10	B25.
ICD-9	078.5
MeSH	D003586

Cytomegalovirus



CMV infection of a lung pneumocyte.

Virus classification	
Group:	Group I (dsDNA)
Family:	*Herpesviridae*
Subfamily:	*Betaherpesvirinae*
Genus:	***Cytomegalovirus***

Cytomegalovirus (from the Greek *cyto-*, "cell", and *-megalo-*, "large") is a herpes viral genus of the Herpesviruses group: in humans it is commonly known as HCMV or **Human Herpesvirus 5** (HHV-5). CMV belongs to the *Betaherpesvirinae* subfamily of *Herpesviridae*, which also includes Roseolovirus. Other herpesviruses fall into the subfamilies of *Alphaherpesvirinae* (including HSV 1 and 2 and varicella) or

Gammaherpesvirinae (including Epstein-Barr virus). All herpesviruses share a characteristic ability to remain latent within the body over long periods.

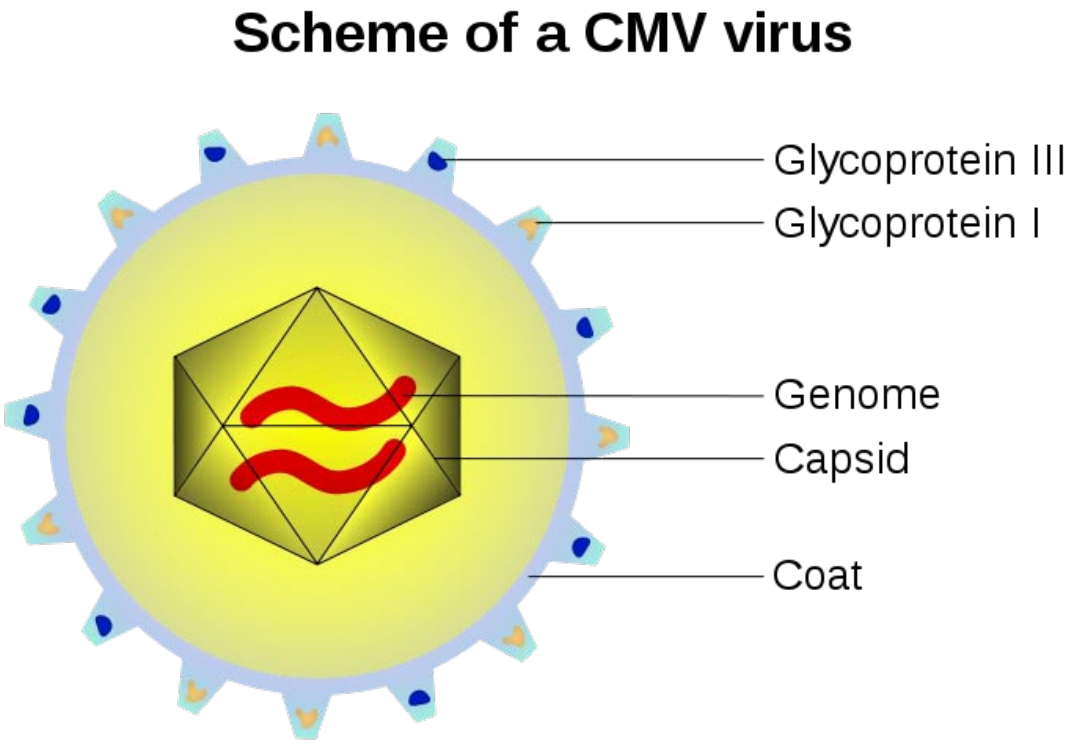
HCMV infections are frequently associated with salivary glands, though they may be found throughout the body. HCMV infection can be life-threatening for people who are immunocompromised (*e.g.* patients with HIV, organ transplant recipients, or neonates). Other CMV viruses are found in several mammal species, but species isolated from animals differ from HCMV in terms of genomic structure, and have not been reported to cause human disease.

HCMV is found throughout all geographic locations and socioeconomic groups, and infects between 50% and 80% of adults in the United States (40% worldwide) as indicated by the presence of antibodies in much of the general population. Seroprevalence is age-dependent: 58.9% of individuals aged 6 and older are infected with CMV while 90.8% of individuals aged 80 and older are positive for HCMV. HCMV is also the virus most frequently transmitted to a developing fetus. HCMV infection is more widespread in developing countries and in communities with lower socioeconomic status and represents the most significant viral cause of birth defects in industrialized countries. CMV "seems to have a large impact on immune parameters in later life and may contribute to increased morbidity and eventual mortality."

Species

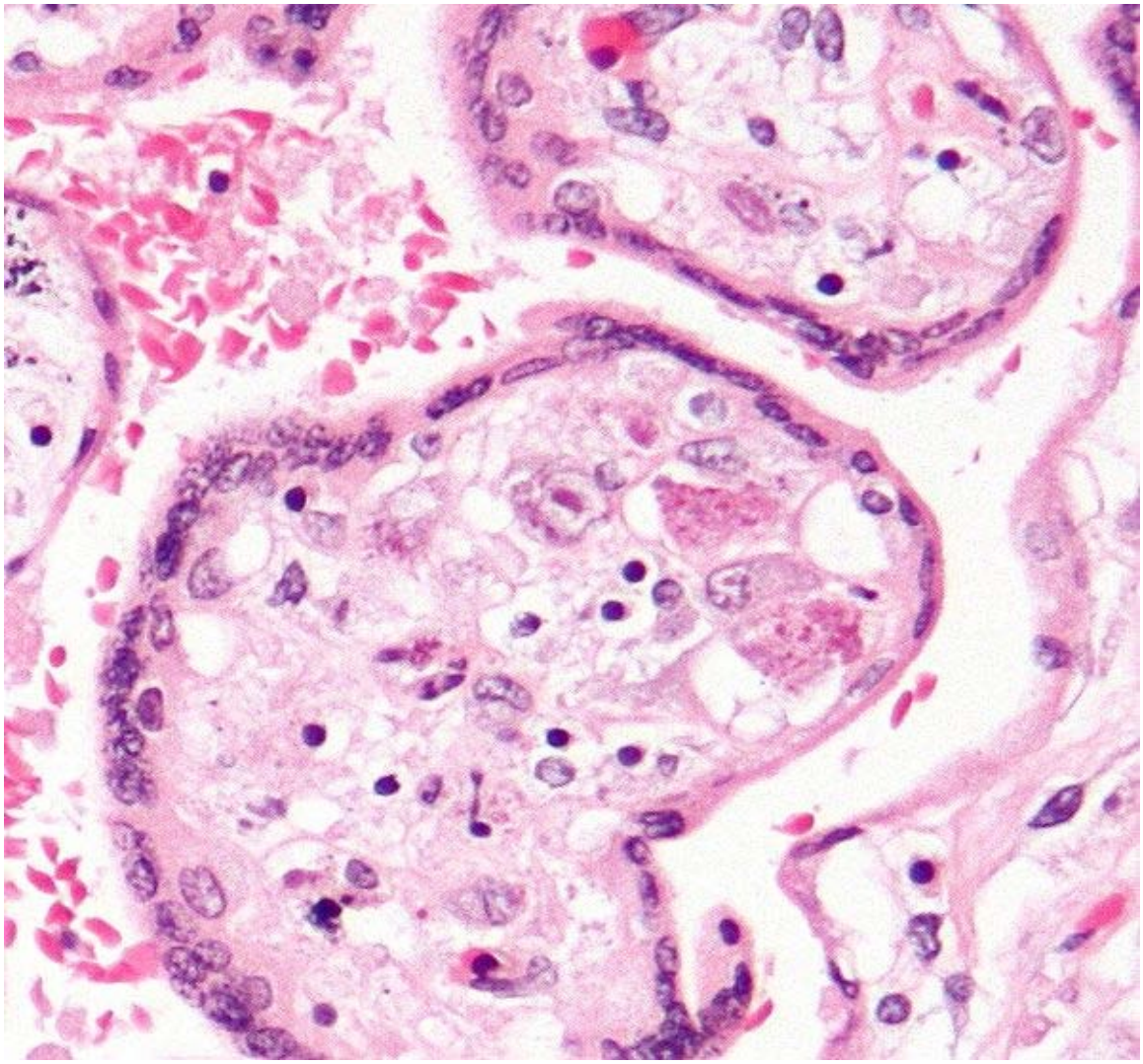
Name	Abv.	Host
Cercopithecine herpesvirus 5	(CeHV-5)	African green monkey
Cercopithecine herpesvirus 8	(CeHV-8)	Rhesus monkey
Human herpesvirus 5	(HHV-5)	Humans
Pongine herpesvirus 4	(PoHV-4)	?
Aotine herpesvirus 1	(AoHV-1)	(Tentative species)
Aotine herpesvirus 3	(AoHV-3)	(Tentative species)

Pathogenesis



Most healthy people who are infected by HCMV after birth have no symptoms. Some develop a syndrome similar to infectious mononucleosis or glandular fever, with prolonged fever, and a mild hepatitis. A sore throat is common. After infection, the virus remains latent in the body for the rest of the person's life. Overt disease rarely occurs unless immunity is suppressed either by drugs, infection or old age. Initial HCMV infection, which often is asymptomatic, is followed by a prolonged, inapparent infection during which the virus resides in T-cells without causing detectable damage or clinical illness.

Infectious CMV may be shed in the bodily fluids of any infected person, and can be found in urine, saliva, blood, tears, semen, and breast milk. The shedding of virus can occur intermittently, without any detectable signs or symptoms.



Micrograph of CMV placentitis. One cell on the image (centre) has the characteristic large nucleus with peri-nuclear clearing. Two cells (centre-left) have the characteristic (cytoplasmic) viral inclusion bodies (small pink globules). H&E stain.

CMV infection can be demonstrated microscopically by the detection of intranuclear inclusion bodies. On H&E staining, the inclusion bodies stain dark pink and are called "owl's eye" inclusion bodies.

HCMV infection is important to certain high-risk groups. Major areas of risk of infection include pre-natal or postnatal infants and immunocompromised individuals, such as organ transplant recipients, persons with leukemia, or those infected with human immunodeficiency virus (HIV). In HIV infected persons, HCMV is considered an *AIDS-defining infection*, indicating that the T-cell count has dropped to low levels.

Lytically replicating virus disrupts the cytoskeleton, causing massive cell enlargement, which is the source of the virus' name.

A study published in 2009 links infection with CMV to high blood pressure in mice, and suggests that the result of CMV infection of blood vessel endothelial cells (EC) in humans is a major cause of atherosclerosis. Researchers also found that when the cells were infected with CMV, they created renin, a protein known to contribute to high blood pressure.

Transmission and prevention

HCMV is transmitted from person to person through bodily fluids. Infection requires close, intimate contact with a person excreting the virus in their saliva, urine, or other bodily fluids. CMV can be transmitted sexually and via breast milk, transplanted organs, and, rarely, from blood transfusions.

Although HCMV is not highly contagious, it has been shown to spread in households and among young children in day care centers. The virus is most often transmitted through infected bodily fluids that come in contact with hands and are then absorbed through the nose or mouth of a susceptible person. This can be minimised by taking care when handling children and items such as diapers; simple hand washing with soap and water is effective in removing the virus from the hands.

HCMV infection without symptoms is common in infants and young children; consequently it is common not to exclude a child known to be infected from school or another institution. Similarly, hospitalized patients are not typically separated or isolated.

Amniocentesis 20 or more weeks into gestation can be done to detect fetal transmission from the mother.

Vaccine

Cytomegalovirus vaccines are still in the research and development stage.

A phase 2 study of a CMV-vaccine published in 2009 indicated an efficacy of 50% - the protection provided was limited, and a number of subjects contracted CMV infection despite vaccination. In one case also congenital CMV was encountered.

CMV diseases

CMV infections are most significant in the perinatal period and in immunocompromised patients.

Pregnancy and congenital infection

HCMV is one of the TORCH infections that lead to congenital abnormalities. These are: toxoplasmosis, rubella, herpes simplex, and cytomegalovirus. Congenital HCMV

infection occurs when the mother suffers a primary infection (or reactivation) during pregnancy.

up to 5/1000 live births are infected. 5% develop multiple handicaps, and develop cytomegalic inclusion disease (with nonspecific signs that resemble rubella. Another 5% later develop cerebral calcification (decreasing IQ levels dramatically, sensorineural deafness and psychomotor retardation.

Immunocompromised adults

Primary CMV infection in patients with weakened immune systems can lead to serious disease. However, a more common problem is reactivation of the latent virus.

Infection with CMV is a major cause of disease and death in immunocompromised patients, including organ transplant recipients, patients undergoing hemodialysis, patients with cancer, patients receiving immunosuppressive drugs, and HIV-infected patients. Exposing immunosuppressed patients to outside sources of CMV should be minimized to avoid the risk of serious infection. Susceptible patients without CMV infection can be infected by receiving infected organs and blood products unless care is taken.

In patients with a depressed immune system, CMV-related disease may be much more aggressive.

Specific disease entities recognised in those people are

- CMV hepatitis, which may cause fulminant liver failure
- cytomegalovirus retinitis (inflammation of the retina, characterised by a "pizza pie appearance" on ophthalmoscopy)
- cytomegalovirus colitis (inflammation of the large bowel)
- CMV pneumonitis
- CMV esophagitis

- polyradiculopathy, transverse myelitis, and subacute encephalitis

Patients without CMV infection who are given organ transplants from CMV-infected donors require prophylactic treatment with valganciclovir (ideally) or ganciclovir, and regular serological monitoring to detect a rising CMV titre; if treated early establishment of a potentially life-threatening infection can be prevented.

Immunocompetent adults

CMV infections can still be of clinical significance in adult immunocompetent populations:

- CMV mononucleosis (some sources reserve "mononucleosis" for EBV only)
- Post-transfusion CMV – similar to CMV mononucleosis

- A 2009 study suggests that CMV infection may be linked to the development of arterial hypertension. Mice fed a high cholesterol diet showed significantly more vascular damage and hypertension when they had been infected with CMV. CMV infection stimulated cytokines – IL6, TNF, and MCP1 – in the infected mice, indicating that the infection led to an inflammatory response in vessels and other tissues. Further, renin and angiotensin II release were increased in these animals as additional factors to lead to hypertension. In humans CMV infection has been demonstrated in the aortic smooth muscle cells from patients with abdominal aortic aneurysms suggesting that CMV infection contributes to vascular disease.

Diagnosis

Most infections with CMV are not diagnosed because the virus usually produces few, if any, symptoms and tends to reactivate intermittently without symptoms. However, persons who have been infected with CMV develop antibodies to the virus, and these antibodies persist in the body for the lifetime of that individual. A number of laboratory tests that detect these antibodies to CMV have been developed to determine if infection has occurred and are widely available from commercial laboratories. In addition, the virus can be cultured from specimens obtained from urine, throat swabs, bronchial lavages and tissue samples to detect active infection. Both qualitative and quantitative polymerase chain reaction (PCR) testing for CMV are available as well, allowing physicians to monitor the viral load of CMV-infected patients.

CMV pp65 antigenemia test is a immunofluorescence based assay which utilizes an indirect immunofluorescence technique for identifying the pp65 protein of cytomegalovirus in peripheral blood leukocytes. The CMV pp65 assay is widely used for monitoring CMV infections and its response to antiviral treatment in patients who are under immunosuppressive therapy and have had renal transplantation surgery as the antigenemia results are obtained about 5 days before the onset of symptomatic CMV disease. The advantage of this assay is the rapidity in providing results in a few hours and that the pp65 antigen determination represents a useful parameter for the physician to initiate antiviral therapy. The major disadvantage of the pp65 assay is that only limited number of samples can be processed per test batch.

CMV should be suspected if a patient has symptoms of infectious mononucleosis but has negative test results for mononucleosis and Epstein-Barr virus, or if they show signs of hepatitis, but has negative test results for hepatitis A, B, and C.

For best diagnostic results, laboratory tests for CMV antibody should be performed by using paired serum samples. One blood sample should be taken upon suspicion of CMV, and another one taken within 2 weeks. A virus culture can be performed at any time the patient is symptomatic. Laboratory testing for antibody to CMV can be performed to determine if a woman has already had CMV infection. However, routine testing of all pregnant women is costly and the need for testing should therefore be evaluated on a case-by-case basis.

Serologic testing

The enzyme-linked immunosorbent assay (or ELISA) is the most commonly available serologic test for measuring antibody to CMV. The result can be used to determine if acute infection, prior infection, or passively acquired maternal antibody in an infant is present. Other tests include various fluorescence assays, indirect hemagglutination, (PCR) and latex agglutination.

An ELISA technique for CMV-specific IgM is available, but may give false-positive results unless steps are taken to remove rheumatoid factor or most of the IgG antibody before the serum sample is tested. Because CMV-specific IgM may be produced in low levels in reactivated CMV infection, its presence is not always indicative of primary infection. Only virus recovered from a target organ, such as the lung, provides unequivocal evidence that the current illness is caused by acquired CMV infection. If serologic tests detect a positive or high titer of IgG, this result should not automatically be interpreted to mean that active CMV infection is present. However, if antibody tests of paired serum samples show a fourfold rise in IgG antibody and a significant level of IgM antibody, meaning equal to at least 30% of the IgG value, or virus is cultured from a urine or throat specimen, the findings indicate that an active CMV infection is present.

Relevance to blood donors

Although the risks discussed above are generally low, CMV assays are part of the standard screening for non-directed blood donation (donations not specified for a particular patient) in the U.S. CMV-negative donations are then earmarked for transfusion to infants or immunocompromised patients. Some blood donation centers maintain lists of donors whose blood is CMV negative due to special demands.

Treatment

Cytomegalovirus Immune Globulin Intravenous (Human) (CMV-IGIV) is an immunoglobulin G (IgG) containing a standardized amount of antibody to Cytomegalovirus (CMV). It may be used for the prophylaxis of cytomegalovirus disease associated with transplantation of kidney, lung, liver, pancreas, and heart.

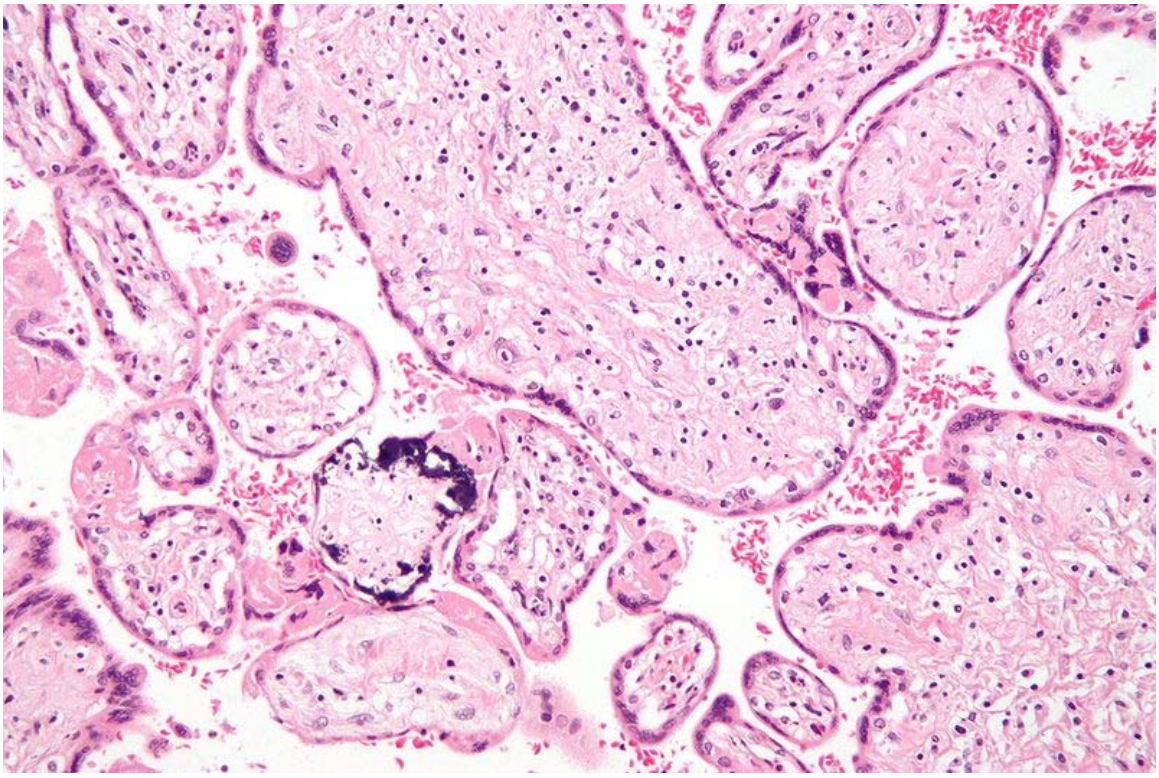
Alone or in combination with an antiviral agent, it has been shown to:

- Reduce the risk of CMV-related disease and death in some of the highest-risk transplant patients
- Provide a measurable long-term survival benefit
- Produce minimal treatment-related side effects and adverse events.

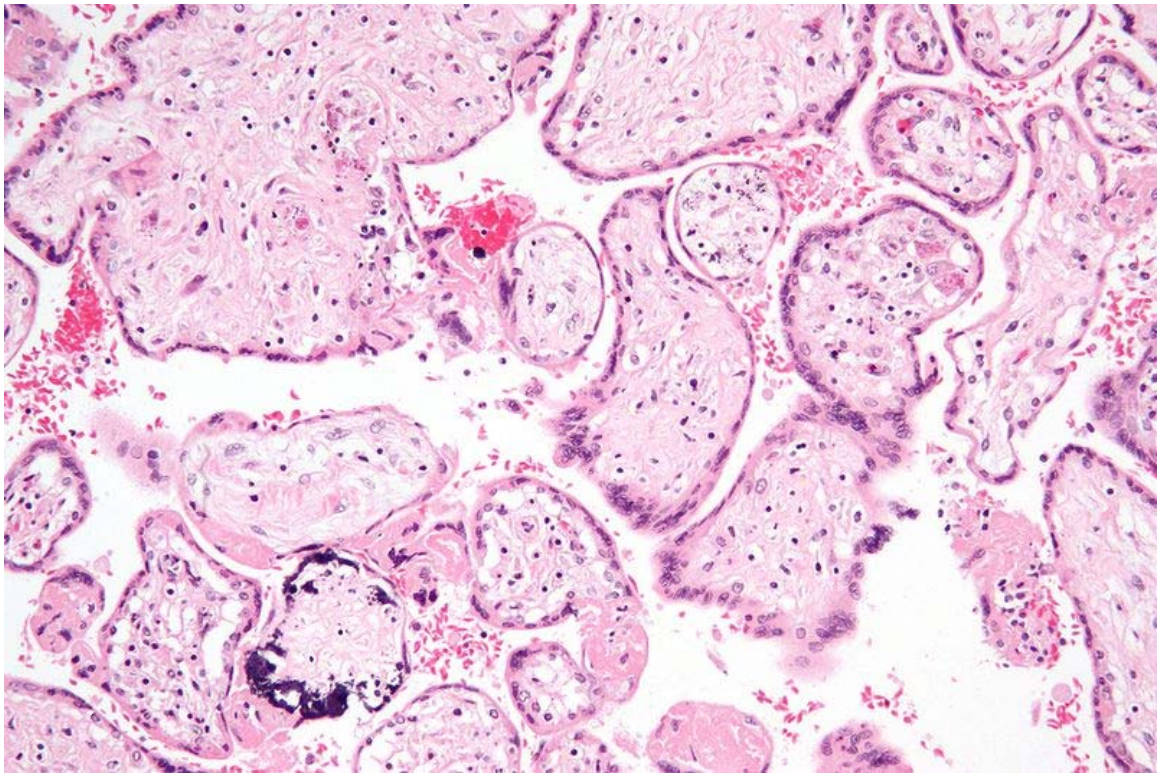
Ganciclovir (Cytovene) treatment is used for patients with depressed immunity who have either sight-related or life-threatening illnesses. Valganciclovir (Valcyte) is an antiviral drug that is also effective and is given orally. The therapeutic effectiveness is frequently compromised by the emergence of drug-resistant virus isolates. A variety of amino acid

changes in the UL97 protein kinase and the viral DNA polymerase have been reported to cause drug resistance. Foscarnet or cidofovir are only given to patients with CMV resistant to ganciclovir, because foscarnet has bad nephrotoxicity, resulting in increased or decreased Ca2+ or P, and decreased Mg2+.

Additional images



Micrograph of a placental infection (CMV placentitis)

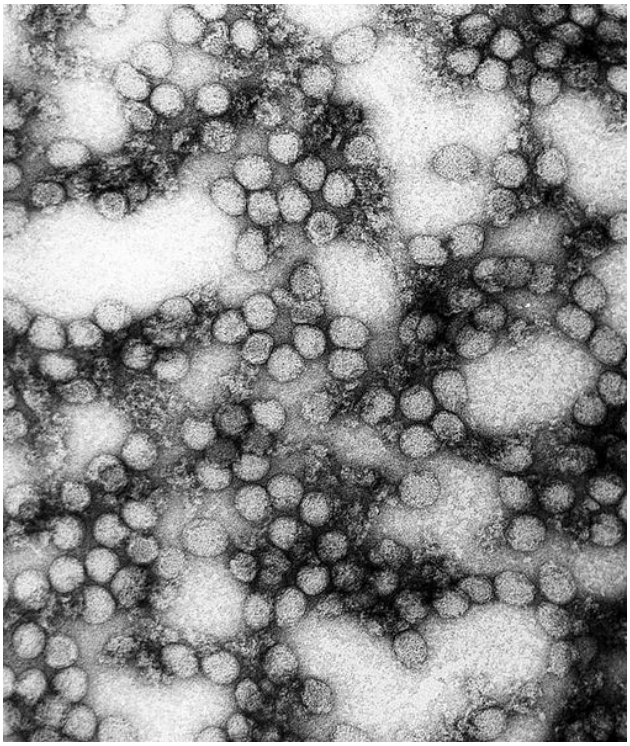


Micrograph of CMV placentitis

Chapter 7

Yellow Fever

Yellow Fever



A TEM micrograph of the yellow fever virus (234,000X magnification)

ICD-10	A95.
ICD-9	060
DiseasesDB	14203
MedlinePlus	001365
eMedicine	med/2432 emerg/645
MeSH	D015004

Yellow fever is an acute viral hemorrhagic disease. The virus is a 40 to 50 nm enveloped RNA virus with positive sense of the Flaviviridae family.

The yellow fever virus is transmitted by the bite of female mosquitoes (the yellow fever mosquito, *Aedes aegypti*, and other species) and is found in tropical and subtropical areas in South America and Africa, but not in Asia. The only known hosts of the virus are primates and several species of mosquito. The origin of the disease is most likely to be Africa, from where it was introduced to South America through the slave trade in the 16th century. Since the 17th century, several major epidemics of the disease have been recorded in the Americas, Africa and Europe. In the 19th century, yellow fever was deemed one of the most dangerous infectious diseases.

Yellow fever presents in most cases with fever, nausea, and pain and it generally subsides after several days. In some patients, a toxic phase follows, in which liver damage with jaundice (giving the name of the disease) can occur and lead to death. Because of the increased bleeding tendency (bleeding diathesis), yellow fever belongs to the group of hemorrhagic fevers. The WHO estimates that yellow fever causes 200,000 illnesses and 30,000 deaths every year in unvaccinated populations; around 90% of the infections occur in Africa.

A safe and effective vaccine against yellow fever has existed since the middle of the 20th century and some countries require vaccinations for travelers. Since no therapy is known, vaccination programs are, along with measures to reduce the population of the transmitting mosquito, of great importance in affected areas. Since the 1980s, the number of cases of yellow fever has been increasing, making it a *reemerging disease*.

Signs and symptoms

Yellow fever begins after an incubation period of three to six days. Most cases only cause a mild infection with fever, headache, chills, back pain, loss of appetite, nausea and vomiting. In these cases the infection lasts only three to four days. 15% of cases enter a second, toxic phase of the disease with recurring fever, this time accompanied by jaundice due to liver damage, as well as abdominal pain. Bleeding in the mouth, the eyes and in the gastrointestinal tract can cause vomitus containing blood (giving the name **black vomit**). The toxic phase is fatal in approximately 20% of cases, making the overall fatality rate for the disease 3% (15% * 20%).

Surviving the infection causes life-long immunity and normally there is no permanent organ damage.

Cause

Yellow fever virus
Virus classification

Group: Group IV ((+)ssRNA)

Family: Flaviviridae

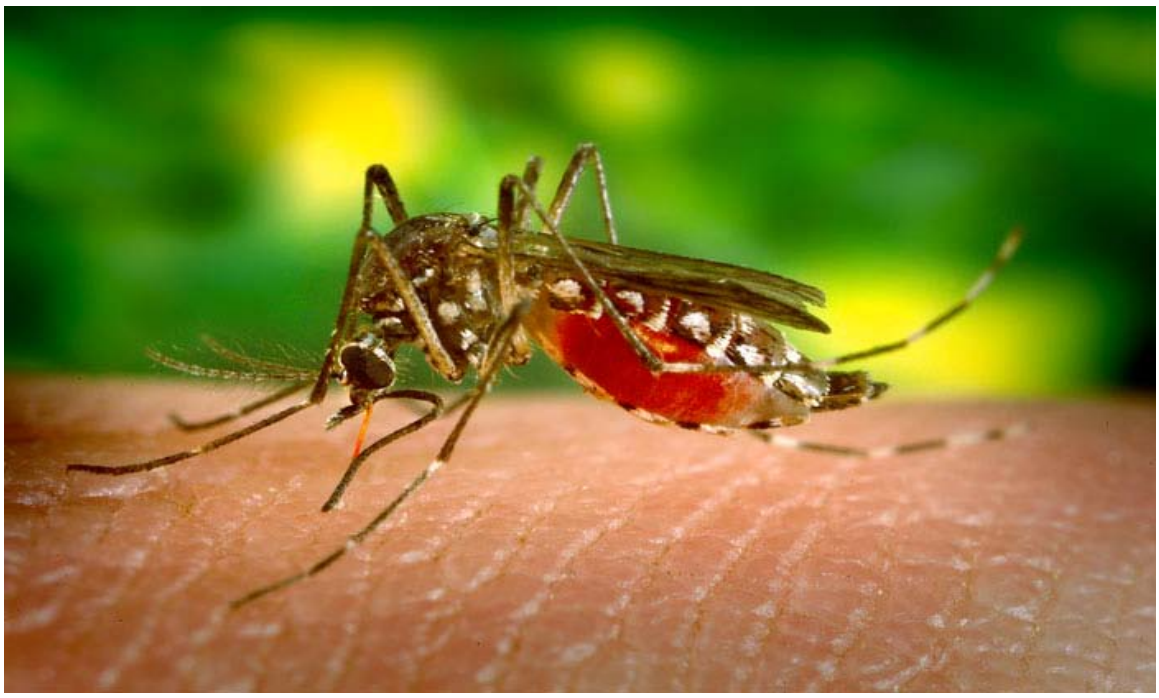
Genus: *Flavivirus*

Species: ***Yellow fever virus***

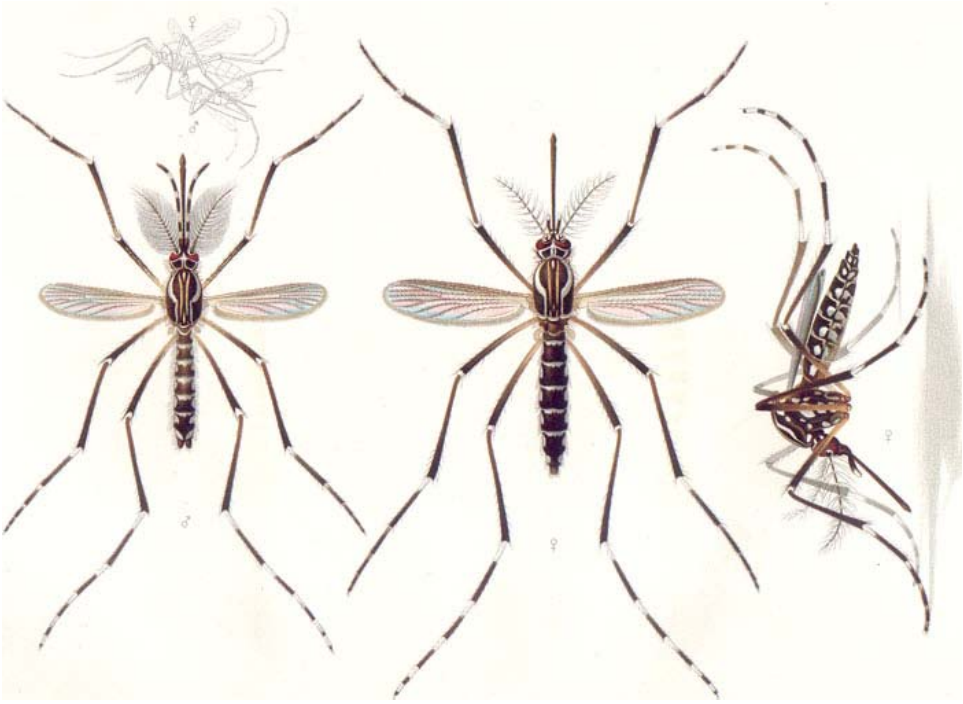
Yellow fever is caused by the yellow fever virus, a 40 to 50 nm wide enveloped RNA virus belonging to the family Flaviviridae. The positive sense single-stranded RNA is approximately 11,000 nucleotides long and has a single open reading frame encoding a polyprotein. Host proteases cut this polyprotein into three structural (C, prM, E) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5); the enumeration corresponds to the arrangement of the protein coding genes in the genome. The viruses infect amongst others monocytes, macrophages and dendritic cells. They attach to the cell surface via specific receptors and are taken up by an endosomal vesicle. Inside the endosome, the decreased pH induces the fusion of the endosomal membrane with the virus envelope. Thus, the capsid reaches the cytosol, decays and releases the genome. Receptor binding as well as membrane fusion are catalyzed by the protein E, which changes its conformation at low pH, which causes a rearrangement of the 90 homodimers to 60 homotrimers.

After entering the host cells, the viral genome is replicated in the rough endoplasmic reticulum (ER) and in the so-called vesicle packets. At first, an immature form of the virus particle is produced inside the ER, whose M-protein is not yet cleaved to its mature form and is therefore denoted as prM (*precursor M*) and forms a complex with protein E. The immature particles are processed in the Golgi apparatus by the host protein furin, which cleaves prM to M. This releases E from the complex which can now take its place in the mature, infectious virion.

Transmission



Aedes aegypti feeding



Adults of the yellow fever mosquito *Aedes aegypti*. The male on the left, females on the right. Only the female mosquito bites can transmit the disease

The yellow fever virus is mainly transmitted through the bite of the yellow fever mosquito *Aedes aegypti*, but other mosquitoes such as the "tiger mosquito" (*Aedes albopictus*) can also serve as a vector for the virus. Like other Arboviruses which are transmitted via mosquitoes, the yellow fever virus is taken up by a female mosquito which sucks the blood of an infected person. Viruses reach the stomach of the mosquito, and if the virus concentration is high enough, the virions can infect epithelial cells and replicate there. From there they reach the haemocoel (the blood system of mosquitoes) and from there the salivary glands. When the mosquito sucks blood the next time, it injects its saliva into the wound, and thus the virus reaches the blood of the bitten person. There are also indications for vertical infection of the yellow fever virus within *A. aegypti*, i.e., the transmission from a female mosquito to her eggs and then larvae. This infection of vectors without a previous blood meal seems to play a role in single, sudden breakouts of the disease.

There are three epidemiologically different infectious cycles, in which the virus is transmitted from mosquitoes to humans or other primates. In the urban cycle, only the yellow fever mosquito *Aedes aegypti* is involved, which is well adapted to urban centres and can also transmit other diseases including Dengue and Chikungunya. The urban cycle is responsible for the major outbreaks of yellow fever that occur in Africa. Except in an outbreak in 1999 in Bolivia, this urban cycle no longer exists in South America and is only present in Africa.

Besides the urban cycle there is, both in Africa and South America, a sylvatic cycle (Forest cycle or Jungle cycle), where *Aedes africanus* (in Africa) or mosquitoes of the genus *Haemagoggus* and *Sabethes* (in South America) serve as a vector. In the jungle, mainly non-human primates get infected; the disease is mostly asymptomatic in African primates. In South America, the sylvatic cycle is currently the only way humans can infect themselves, which explains the low incidence of yellow fever cases on this continent. People who become infected in the jungle can carry the virus to urban centres, where *Aedes aegypti* acts as a vector. It is because of this sylvatic cycle that yellow fever cannot be eradicated.

In Africa there is a third infectious cycle, also known as savannah cycle or intermediate cycle, which occurs between the jungle and urban cycle. Different mosquitoes of the genus *Aedes* are involved. In recent years this is the most common form of yellow fever seen in Africa.

Pathogenesis

After transmission of the virus from a mosquito the viruses replicate in the lymph nodes and infect dendritic cells in particular. From there they reach the liver and infect hepatocytes (probably indirectly via Kupffer cells), which leads to eosinophilic degradation of these cells and to the release of cytokines. Necrotic masses (Councilman bodies) appear in the cytoplasm of hepatocytes.

When the disease takes a deadly course, a cardiovascular shock and multi organ failure with strongly increased cytokine levels (cytokine storm) follow.

Diagnosis

Yellow fever is a clinical diagnosis, which often relies on the whereabouts of the diseased person during the incubation time. Mild courses of the disease can only be confirmed virologically. Since also mild courses of yellow fever can significantly contribute to regional outbreaks, every suspected yellow fever has to be treated seriously (six to ten days after leaving the affected area symptoms of fever, pain, nausea and vomiting).

If yellow fever is suspected, the virus can be confirmed until six to ten days after the illness. A direct confirmation can be obtained by Reverse transcription polymerase chain reaction where the genome of the virus is amplified. Another direct approach is the isolation of the virus and its growth in cell culture using blood plasma; this can take one to four weeks.

Serologically an enzyme linked immunosorbent assay during the acute phase of the disease using specific IgM against yellow fever or an increase in specific IgG-titer (compared to an earlier sample) can confirm yellow fever. Together with clinical symptoms, the detection of IgM or a fourfold increase in IgG-titer is considered sufficient indication for yellow fever. Since these tests can cross-react with other Flaviviruses, like Dengue virus, these indirect methods can never prove yellow fever infection. Liver biopsy can verify inflammation and necrosis of hepatocytes and detect viral antigens. Because of the bleeding tendency of yellow fever patients, a biopsy is only advisable *post mortem* to confirm the cause of death.

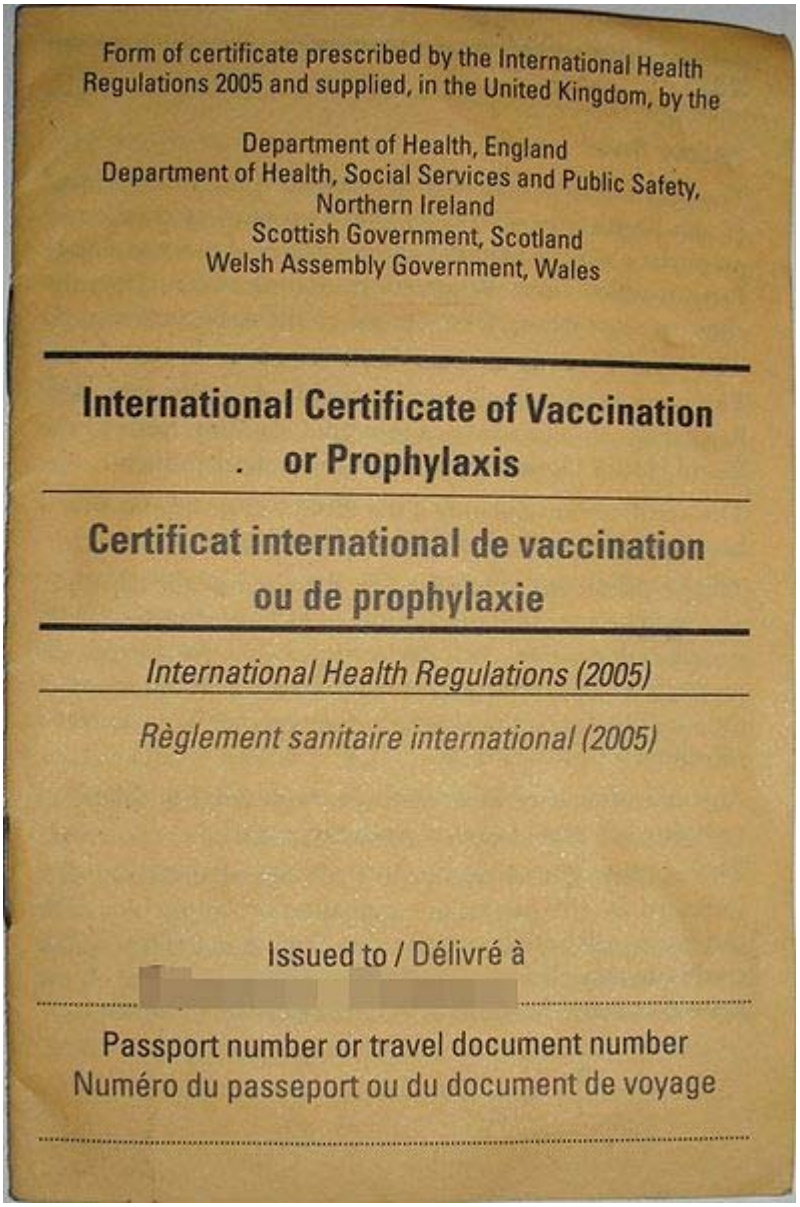
In a differential diagnosis, infections with yellow fever have to be distinguished from other feverish illnesses like malaria. Other viral hemorrhagic fever, such as Ebola virus, Lassa virus, Marburg virus or Junin virus have to be excluded as cause.

Prevention

Personal prevention of yellow fever includes vaccination as well as avoidance of mosquito bites in areas where yellow fever is endemic. Institutional measures for prevention of yellow fever include vaccination programmes and measures of controlling mosquitoes.

Vaccination

Injection of protective vaccination into the deltoid muscle


Form of certificate prescribed by the International Health Regulations 2005 and supplied, in the United Kingdom, by the

Department of Health, England
Department of Health, Social Services and Public Safety, Northern Ireland
Scottish Government, Scotland
Welsh Assembly Government, Wales

International Certificate of Vaccination or Prophylaxis

Certificat international de vaccination ou de prophylaxie

International Health Regulations (2005)

Règlement sanitaire international (2005)

Issued to / Délivré à

Passport number or travel document number
Numéro du passeport ou du document de voyage

The cover of a certificate that confirms that the holder has been vaccinated against yellow fever

For journeys into affected areas, vaccination is highly recommended since mostly non-native people are affected by severe cases of yellow fever. The protective effect is established 10 days after vaccination in 95% of the vaccinated people and lasts for at least 10 years (even 30 years later, 81% of patients retained the immunity). The attenuated live vaccine (stem 17D) was developed in 1937 by Max Theiler from a diseased patient in Ghana and is produced in chicken eggs. WHO recommends routine vaccinations for people living in endemic areas between the 9th and 12th month after birth.

In about 20% of all cases, mild, flu-like symptoms may develop. In rare cases (less than one in 200,000 to 300,000), the vaccination can cause YEL-AVD (*yellow fever vaccine-associated viscerotropic disease*), which is fatal in 60% of all cases. It is probably due to a genetic defect in the immune system. But in some vaccination campaigns, a 20 fold higher incidence rate has been reported. Age is an important risk factor; in children the complication rate is less than one case per 10 million vaccinations. Another possible side effect is an infection of the nervous system that occurs in one in 200,000 to 300,000 of all cases, causing YEL-AND (*yellow fever vaccine-associated neurotropic disease*), which can cause meningoencephalitis and is less than 5% of all cases fatal.

In 2009, the largest mass vaccination against yellow fever commenced in West Africa, specifically Benin, Liberia and Sierra Leone. When it is completed in 2015, more than 12 million people will have been vaccinated against the disease. According to the World Health Organization, the mass vaccination cannot eliminate yellow fever because of the massive number of infected mosquitoes in urban areas of the target countries, but it will significantly reduce the number of people infected. However, the WHO plans to continue the vaccination campaign in another five African countries—Central African Republic, Ghana, Guinea, Ivory Coast and Nigeria—and claimed that approximately 160 million people in the continent could be at risk unless the organization acquires additional funding.

Compulsory vaccination

Some countries in Asia are theoretically in danger of yellow fever epidemics (mosquitoes with the capability to transmit yellow fever and susceptible monkeys are present), even though the disease does not yet occur there. To prevent introduction of the virus, some countries demand previous vaccination of foreign visitors, if they have passed through yellow fever areas. Vaccination has to be proven in a vaccination certificate which is valid 10 days after the vaccination and lasts for 10 years. A list of the countries which require yellow fever vaccination is published by the WHO. If the vaccination cannot be conducted for some reasons, dispensation is possible. In this case an exemption certificate issued by a WHO approved vaccination center is required.

Even though 32 of 44 countries where yellow fever occurs endemically do have vaccination programmes, in many of these countries fewer than 50% of their population is vaccinated.

Vector control

Information campaign for prevention of Dengue and yellow fever in Paraguay

Besides vaccination, control of the yellow fever mosquito *Aedes aegypti* is of major importance, especially because the same mosquito can also transmit Dengue and Chikungunya disease. *Aedes aegypti* breeds preferentially in water, for example in installations by inhabitants of areas with precarious drinking water supply, or in domestic waste; especially tires, cans and plastic bottles. Especially in proximity to urban centres of developing countries these conditions are very common and make a perfect habitat for *Aedes aegypti*. Two strategies are employed to fight the mosquito:

One approach is to kill the developing larva. Measures are taken to reduce water build-up (the habitat of the larva), and larvicides are used as well as larva-eating fish and copepods, which reduce the number of larva and thus indirectly the number of disease-transmitting mosquitoes. For many years, copepods of the genus *Mesocyclops* have been used in Vietnam for fighting Dengue fever (yellow fever does not occur in Asia), with the effect that in the affected areas no cases of Dengue fever have occurred since 2001. Similar mechanisms are probably also effective against yellow fever. Pyriproxyfen is recommended as a chemical larvicide, mainly because it is safe for humans and effective even in small doses.

Besides larva, the adult yellow fever mosquitoes are also targeted. The curtains and lids of water tanks are sprayed with insecticides. Spraying insecticides inside houses is another measure, although it is not recommended by the WHO. Similar to the malaria carrier, the *Anopheles* mosquito, insecticide treated mosquito nets are used successfully against *Aedes aegypti*.

Treatment

For yellow fever there is, like for all diseases caused by Flaviviruses, no causative cure. Hospitalization is advisable and intensive care may be necessary because of rapid deterioration in some cases. Different methods for acute treatment of the disease have been shown to not be very successful; passive immunisation after emergence of symptoms is probably without effect. Ribavirin and other antiviral drugs as well as treatment with interferons do not have a positive effect in patients. A symptomatic treatment includes rehydration and pain relief with drugs like paracetamol. Acetylsalicylic acid (for example *Aspirin*) should not be given because of its anticoagulant effect, which can be devastating in the case of inner bleeding that can occur with yellow fever.

Epidemiology

Endemic range of yellow fever in South America (2009)

Endemic range of yellow fever in Africa (2009)

Yellow fever is endemic in tropical and subtropical areas of South America and Africa. Even though the main vector *Aedes aegypti* also occurs in Asia, in the Pacific and the Middle East, yellow fever does not occur in these areas; the reason for this is unknown. Worldwide there are about 600 million people living in endemic areas and the official estimations of the WHO amount to 200,000 cases of disease and 30,000 deaths a year; the number of officially reported cases is far lower. An estimated 90% of the infections occur on the African continent. In 2008, the largest number of cases was recorded in Togo.

Phylogenetic analysis identified seven genotypes of yellow fever viruses, and it is assumed that they are differently adapted to humans and to the vector *Aedes aegypti*. Five genotypes occur solely in Africa, and is assumed that the West Africa–genotype I is especially virulent or infectious, because this type is often associated with major outbreaks of yellow fever. In South America two genotypes have been identified.

History

Carlos Finlay

Walter Reed

The evolutionary origins of yellow fever most likely lie in Africa. It is thought that the virus originated in East or Central Africa and spread from there to West Africa. The virus as well as the vector *A. aegypti* were probably brought to South America by ship after 1492. The first probable outbreak of the disease was in 1648 in Yucatan, where the illness was termed *xekik* (black vomit). At least 25 major outbreaks followed, such as in Philadelphia 1793, where several thousand people died and the American administration as well as George Washington had to flee the city. Yellow fever epidemics in North America caused some 100,000-150,000 deaths. Major outbreaks also occurred in Europe, e.g. in 1821 in Barcelona with several thousand victims. In 1858, one church in Charleston, South Carolina, St. Matthew's German Evangelical Lutheran Church, suffered 308 yellow fever deaths which reduced the congregation by half. In 1873, Shreveport Louisiana lost almost one-quarter of it's total population to Yellow Fever. On

Nov. 15, 1873, local news paper, the Times, printed a "List of the Dead," that numbered almost 800. In 1878, about 20,000 people died in an epidemic in the Mississippi River Valley and the last major outbreak in the US occurred in 1905 in New Orleans. In colonial times, West Africa became known as "the white man's grave" because of malaria and yellow fever.

Carlos Finlay, a Cuban doctor and scientist, first proposed in 1881 that yellow fever might be transmitted by mosquitoes rather than direct human contact. Since the losses from yellow fever in the invasion of Cuba in the 1890s were thirteenfold higher than the losses due to military operations, further experiments were conducted by a team under Walter Reed that successfully proved the ″Mosquito Hypothesis″. Yellow fever was thus the first virus shown to be transmitted by mosquitoes. The physician William Gorgas then applied these insights and eradicated yellow fever from Havana, and fought yellow fever during the construction of the Panama Canal – after a French effort to build the canal had failed due to, among other reasons, the high incidence of yellow fever and malaria.

Although Dr. Reed received much of the credit in history books for "beating" yellow fever, Reed himself credited Dr. Finlay with the discovery of the yellow fever vector, and thus how it might be controlled. Dr. Reed often cited Finlay's papers in his own articles and gave him credit for the discovery, even in his personal correspondence. The acceptance of Finlay's work was one of the most important and far-reaching effects of the Walter Reed Commission of 1900. Applying methods first suggested by Finlay, yellow fever was eradicated in Cuba and later in Panama, allowing completion of the Panama Canal.

In 1927, the yellow fever virus was isolated in West Africa, which led to the development of two vaccines in the 1930s. The vaccine 17D was developed by the South African microbiologist Max Theiler at the Rockefeller Institute. Following the work of Ernest Goodpasture, he used chicken eggs to culture the virus and won a Nobel Prize for this achievement in 1951. A French team developed the vaccine FNV (*French neurotropic vaccine*), which was extracted from mouse brain tissue – but since it was associated with a higher incidence of encephalitis, FNV was not recommended after 1961. 17D on the other hand is still in use and over 400 million doses have been distributed. Little has been invested in the development of new vaccines, and the 60-year-old technology might be too slow to stop a yellow fever epidemic. Newer vaccines based on vero cells are in development and should replace 17D at some point.

Using vector control and strict vaccination programs, the urban cycle of yellow fever has been eradicated from South America and since 1943 – apart from an urban outbreak in Santa Cruz de la Sierra (Bolivia) – there has been no yellow fever transmission by *A. aegypti* reported. Since the 1980s, the number of yellow fever cases has been increasing again and *A. aegypti* has returned to the urban centres of South America; partly because the vector control program was abandoned. Even though no new urban cycle has yet established itself, it is feared that this might happen at any point. An outbreak in Paraguay in 2008 was first thought to be urban in nature, but this turned out to not be the case.

In Africa on the other hand, virus eradication programs have mostly used vaccination, but have been unsuccessful since they could not break the sylvatic cycle. Since measures to fight yellow fever have been neglected, with few countries establishing regular vaccination programs, the virus could spread again.

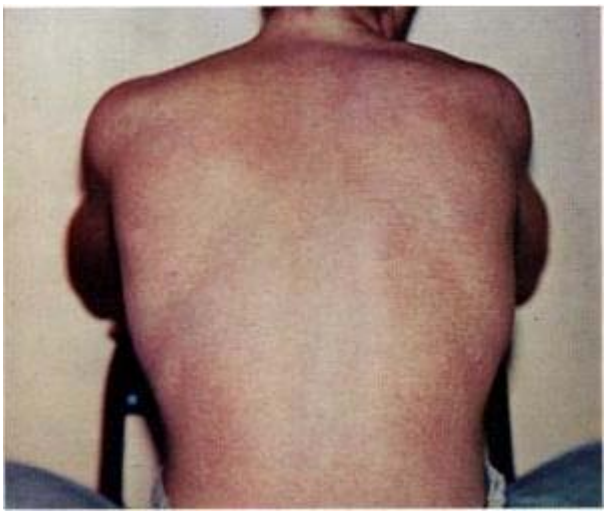
Research

In the hamster model of yellow fever, early administration of the antiviral ribavirin is an effective early treatment of many pathological features of the disease. Ribavirin treatment during the first five days after virus infection improved survival rates, reduced tissue damage in target organs (liver and spleen), prevented hepatocellular steatosis, and normalised alanine aminotransferase (a liver damage marker) levels. The results of this study suggest that ribavirin may be effective in the early treatment of yellow fever, and that its mechanism of action in reducing liver pathology in yellow fever virus infection may be similar to that observed with ribavirin in the treatment of hepatitis C, a virus related to yellow fever. Because ribavirin had failed to improve survival in a virulent primate (rhesus) model of yellow fever infection, it had been previously discounted as a possible therapy.

Chapter 8

Dengue Fever

Dengue fever



The typical rash seen in dengue fever

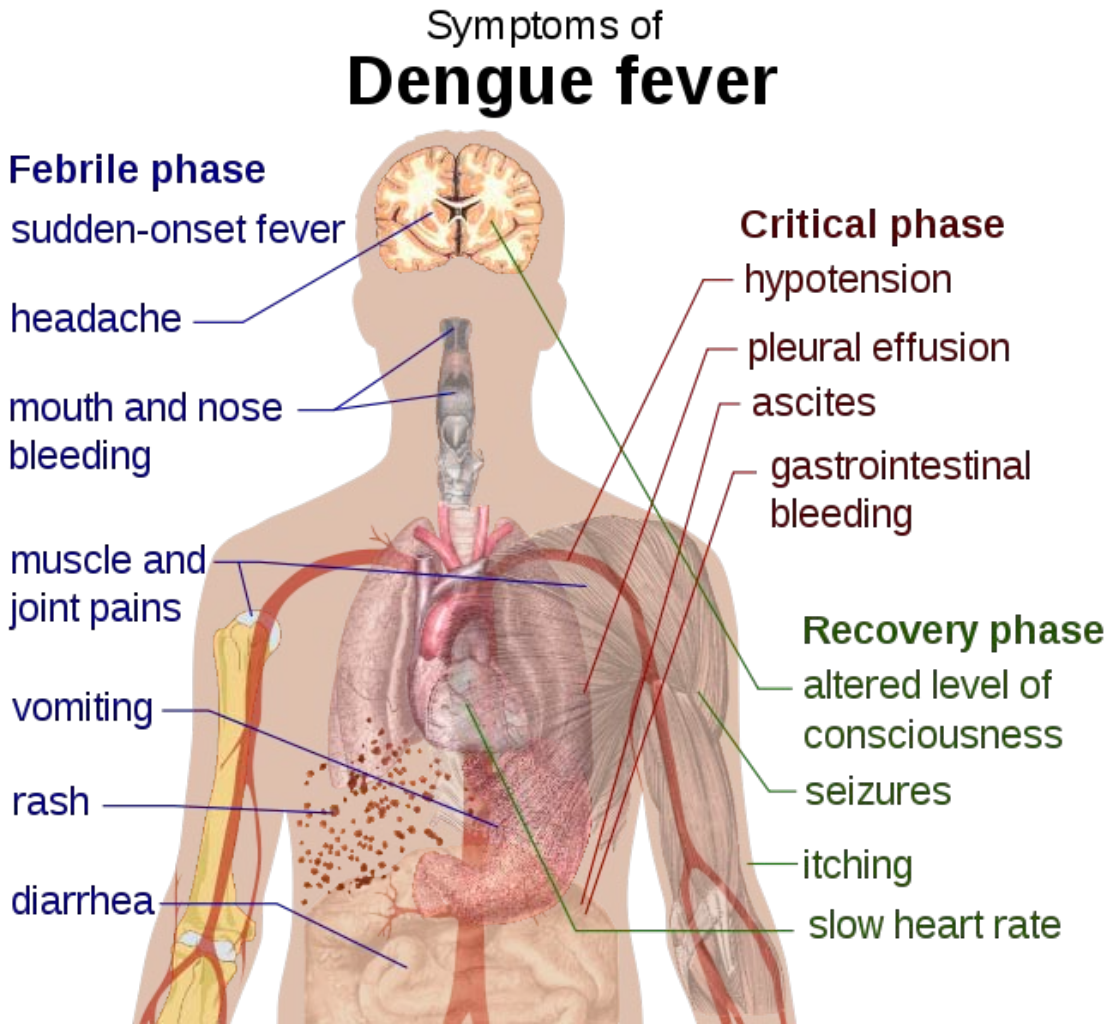
ICD-10	A90.
ICD-9	061
DiseasesDB	3564
MedlinePlus	001374
eMedicine	med/528
MeSH	*C02.782.417.214*

Dengue fever also known as **breakbone fever**, is an acute febrile infectious disease caused by the dengue virus. Typical symptoms include headache, a characteristic skin rash, and muscle and joint pains; in a small proportion the disease progresses to life-threatening complications such as **dengue hemorrhagic fever** (which may lead to severe hemorrhage) and **dengue shock syndrome** (where a very low blood pressure can cause organ dysfunction).

Dengue is usually transmitted by the mosquito *Aedes aegypti*, and rarely *Aedes albopictus*. The virus exists in four different types, and an infection with one type usually gives lifelong immunity to that type, but only short-term immunity to the others. There is currently no available vaccine, but measures to reduce the habitat and the number of mosquitoes, and limiting exposure to bites, are used to decrease the incidence of dengue.

Treatment of acute dengue is supportive, using either oral or intravenous rehydration for mild or moderate disease, and intravenous fluids and blood transfusions for more severe cases. The rate of infection has increased dramatically over the last 50 years, with around 50–100 million people being infected yearly. A global disease, dengue is currently endemic in more than 110 countries. Early descriptions of the condition date from 1779, and its viral cause and the transmission were elucidated in the early 20th century. Dengue has become a worldwide problem since the Second World War.

Signs and symptoms



Schematic depiction of the symptoms of dengue fever

People infected with dengue virus are commonly asymptomatic or only have mild symptoms such as an uncomplicated fever. Others have much more severe illness, and in a small proportion it is life-threatening. The incubation period (time between exposure and onset of symptoms) ranges from 3–14 days, but most often it is 4–7 days. This means that travellers returning from endemic areas are unlikely to have dengue if fever or other symptoms start more than 14 days after arriving home. Children often experience symptoms similar to those of the common cold and gastroenteritis (vomiting and diarrhea), but are more susceptible to the severe complications.

Clinical course

The characteristic symptoms of dengue are: a sudden-onset fever, headache (typically behind the eyes), muscle and joint pains, and a rash; the alternative name for dengue, "break-bone fever", comes from the associated muscle and joints pains. The course of infection is divided into three phases: febrile, critical, and recovery.

The febrile phase involves high fevers, frequently over 40 °C (104 °F) and associated with generalized pain and a headache; this usually lasts 2–7 days. Flushed skin and some small red spots called petechia, which are caused by broken capillaries, may occur at this point, as may some mild bleeding from mucous membranes of the mouth and nose.

The critical phase, if it occurs, follows the resolution of the high fever and typically lasts one to two days. During this phase there may be significant fluid accumulation in the chest and abdominal cavity due to increased capillary permeability and leakage. This leads to depletion of fluid from the circulation and decreased blood supply to vital organs. During this phase, organ dysfunction and severe bleeding (typically from the gastrointestinal tract) may occur. Shock and hemorrhage occur in less than 5% of all cases of dengue but those who have previously been infected with other serotypes of dengue virus ("secondary infection") have an increased risk of this.

The recovery phase occurs next, with resorption of the leaked fluid into the bloodstream. This usually occurs over a period of two to three days. The improvement is often striking, but there may be severe itching and a slow heart rate. It is during this stage that a fluid overload state may occur, which if it affects the brain may reduce the level of consciousness or cause seizures.

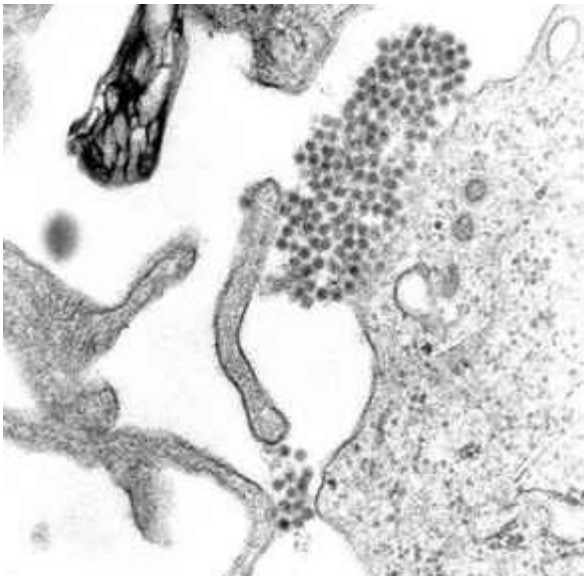
Associated problems

Dengue may occasionally affect several other body systems. This may be either in isolation or along with the classic dengue symptoms. A decreased level of consciousness occurs in 0.5–6% of severe cases. This may be caused by infection of the brain by the virus or indirectly due to impairment of vital organs, for example, the liver.

Other neurological disorders has been reported in the context of dengue, such as transverse myelitis and Guillain-Barré syndrome. Infection of the heart and acute liver failure are among the rarer complications of dengue.

Cause

Virology



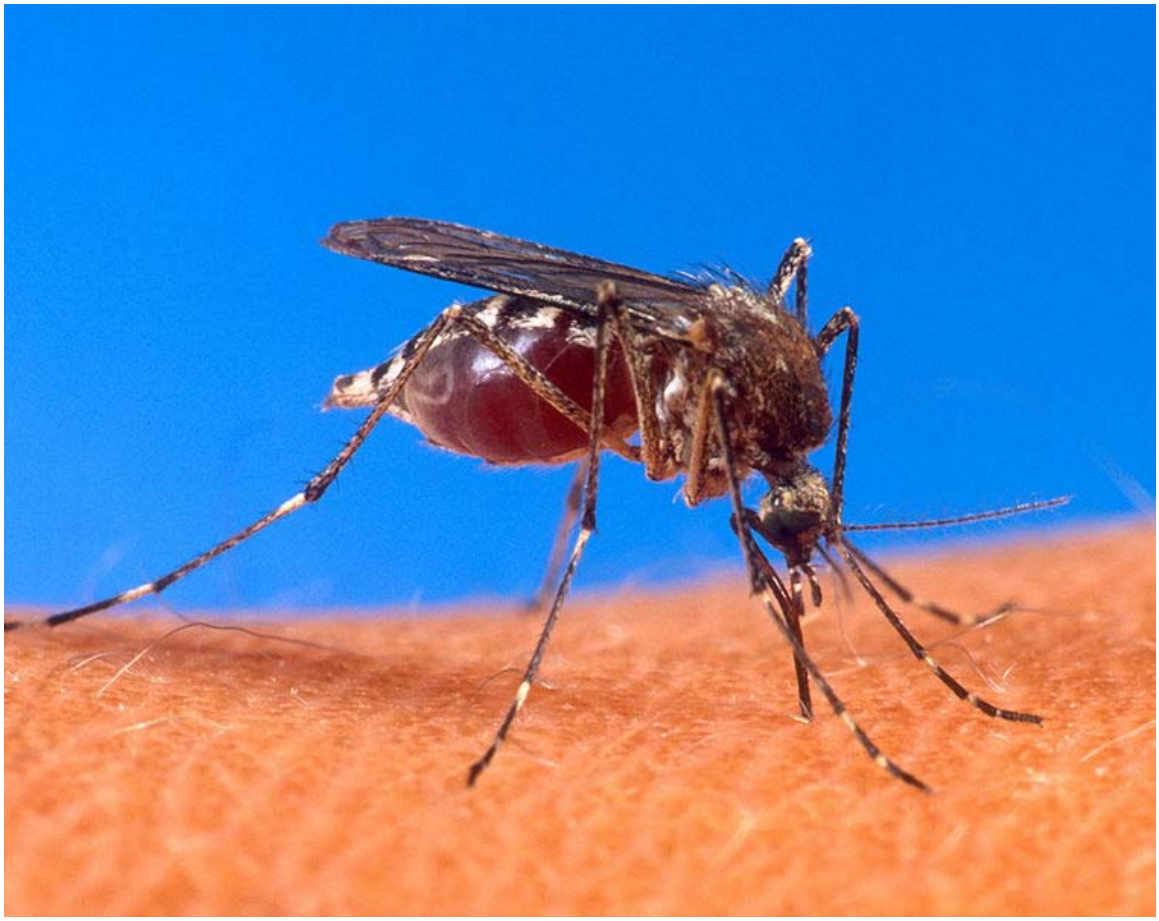
A TEM micrograph showing dengue virus virions (the cluster of dark dots near the center)

Dengue fever virus (DENV) is an RNA virus of the family *Flaviviridae*; genus *Flavivirus*. Other members of the same family include yellow fever virus, West Nile virus, St. Louis encephalitis virus, Japanese encephalitis virus, tick-borne encephalitis virus, Kyasanur forest disease virus, and Omsk hemorrhagic fever virus. Most are transmitted by arthropods (mosquitoes or ticks), and are therefore also referred to as arboviruses (arthropod-borne viruses).

The dengue virus genome (genetic material) contains of about 11,000 nucleotide bases, which code for the three different types of protein molecules that form the virus particle (C, prM and E) and seven other types of protein molecules (NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5) that are only found in infected host cells and are required for replication of the virus. There are four strains of the virus, which are called serotypes, and these are referred to as DENV-1, DENV-2, DENV-3 and DENV-4. All four serotypes can cause the full spectrum of disease. Infection with one serotype is believed to produce lifelong immunity to it but only short term protection against the others.

The severe complications on secondary infection seem to occur particularly if someone previously exposed to serotype DENV-1 then contracts serotype DENV-2 or serotype DENV-3, or if someone previously exposed to type DENV-3 then acquires DENV-2.

Transmission



The mosquito *Aedes aegypti* feeding off a human host

Dengue virus is primarily transmitted by *Aedes* mosquitoes, particularly *A. aegypti*. These mosquitoes usually live between the latitudes of 35 degrees North and 35 degrees South below an elevation of 1,000 metres (3,300 ft). They bite primarily during the day. Other mosquito species—*A. albopictus*, *A polynesiensis* and several *A. scutellaris*—may also transmit the disease. Humans are the primary host of the virus, but it may also circulate in nonhuman primates. An infection may be acquired via a single bite. A mosquito that takes a blood meal from a person infected with dengue fever becomes itself infected with the virus in the cells lining its gut. About 8–10 days later, the virus spreads to other tissues including the mosquito's salivary glands and is subsequently released into its saliva. The virus seems to have no detrimental effect on the mosquito, which remains infected for life. *Aedes aegypti* prefers to lay its eggs in artificial water containers and tends to live in close proximity to humans, and has a preference for feeding off them rather than other vertebrates.

Dengue may also be transmitted via infected blood products and through organ donation. In countries such as Singapore, where dengue is endemic, the risk is estimated to be between 1.6 and 6 per 10,000 transfusions. Vertical transmission (from mother to child)

during pregnancy or at birth has been observed. Other person-to-person modes of transmission have been reported, but are very unusual.

Predisposition

Severe disease is more common in babies and young children, and in contrast to many other infections it is more common in children that are relatively well nourished. Women are more at risk than men. Dengue may be life-threatening in people with chronic diseases such as diabetes and asthma.

It is thought that polymorphisms (normal variations) in particular genes may increase the risk of severe dengue complications. Examples include the genes coding for the proteins known as TNFα, mannan-binding lectin, CTLA4, TGFβ, DC-SIGN, and particular alleles of human leukocyte antigen. A genetic abnormality common in Africans, known as glucose-6-phosphate dehydrogenase deficiency, appears to increase the risk. Polymorphisms in the genes for the vitamin D receptor and FcγR seem to offer protection.

Mechanism

When a mosquito carrying DENV bites a person, the virus enters the skin together with the mosquito's saliva. It binds to and enters white blood cells, and reproduces inside the cells while they move throughout the body. The white blood cells respond by producing a number of signalling proteins (such as interferon) that are responsble for many of the symptoms, such as the fever, the flu-like symptoms and the severe pains. In severe infection, the virus production inside the body is much increased, and many more organs (such as the liver and the bone marrow) can be affected, and fluid from the bloodstream leaks through the wall of small blood vessels into body cavities. As a result, less blood circulates in the blood vessels, and the blood pressure becomes so low that it cannot supply sufficient blood to vital organs. Furthermore, dysfunction of the bone marrow leads to reduced numbers of platelets, which are necessary for effective blood clotting; this increases the risk of bleeding, the other major complication of dengue.

Viral reproduction

After entering the skin, DENV binds to Langerhans cells (a population of dendritic cells in the skin that identifies pathogens). The virus enters the cells through binding between viral proteins and membrane proteins on the Langerhans cell, specifically the C-type lectins called DC-SIGN, mannose receptor and CLEC5A. DC-SIGN, a non-specific receptor for foreign material on dendritic cells, seems to be the main one. The dendritic cell moves to the nearest lymph node. Meanwhile, the virus genome is replicated in membrane-bound vesicles on the cell's endoplasmic reticulum, where the cell's protein synthesis apparatus produces new viral proteins, and the viral RNA is copied. Immature virus particles are transported to the Golgi apparatus, the part of the cell where the some of the proteins receive necessarily sugar chains (glycoproteins). The now mature new

viruses bud on the surface of the infected cell and are released by exocytosis. They are then able enter other white blood cells (such as monocytes and macrophages).

The initial reaction of infected cells is to produce the interferon, a cytokine that raises a number of defenses against viral infection through the innate immune system by augmenting the production of a large group of proteins (mediated by the JAK-STAT pathway). Some serotypes of DENV appear to have mechanisms to slow down this process. Interferon also activates the adaptive immune system, which leads to the generation of antibodies against the virus as well as T cells that directly attack any cell infected with the virus. Various antibodies are generated; some bind closely to the viral proteins and target them for phagocytosis (ingestion by specialized cells) and destruction, but some bind the virus less well and appear instead to deliver the virus into a part of the phagocytes where it is not destroyed but is able to replicate further.

Severe disease

It is not entirely clear why secondary infection with a different strain of DENV places people at risk of dengue hemorrhagic fever and dengue shock syndrome. The most widely accepted hypothesis is that of antibody-dependent enhancement (ADE). The exact mechanism behind ADE is unclear. It may be caused by poor binding of non-neutralizing antibodies and delivery into the wrong compartment of white blood cells that have ingested the virus for destruction. There is a suspicion that ADE is not the only mechanism underlying severe dengue-related complications, and various lines of research have implied a role for T cells and soluble factors (such as cytokines and the complement system).

Severe disease is marked by two problems: dysfunction of endothelium (the cells that line blood vessels) and disordered coagulation (blood clotting). Endothelial dysfunction leads to the leakage of fluid from the blood vessels into the chest and abdominal cavities, while coagulation disorder is responsible for the bleeding complications. Higher levels of virus in the blood and involvement of other organs (such as the bone marrow and the liver) are associated with more severe disease. Cells in the affected organs die, leading to the release of cytokines and activation of both coagulation and fibrinolysis (the opposing systems of blood clotting and clot degradation). These alterations together lead to both endothelial dysfunction and coagulation disorder.

Diagnosis

General

The diagnosis of dengue is typically made clinically, on the basis of reported symptoms and physical examination; this applies especially in endemic areas. Early disease can however be difficult to differentiate from other viral infections. A probable diagnosis is based on the findings of fever plus two of the following: nausea and vomiting, rash, generalized pains, low white blood cell count, positive tourniquet test, or any warning sign in someone who lives in an endemic area. Warning signs typically occur before the

onset of severe dengue. The tourniquet test, which is particularly useful in settings where no laboratory investigations are readily available, involves the application of a blood pressure cuff for five minutes, followed by the counting of any petechial hemorrhages; a higher number makes a diagnosis of dengue more likely. It may be difficult to distinguish dengue fever and chikungunya, a similar viral infection that shares many symptoms and occurs in similar parts of the world to dengue. Often, investigations are performed to exclude other conditions that cause similar symptoms, such as malaria, leptospirosis, typhoid fever, and meningococcal disease.

The earliest change detectable on laboratory investigations is a low white blood cell count, which may then be followed by low platelets and metabolic acidosis. Plasma leakage may result in hemoconcentration (as indicated by a rising hematocrit) and hypoalbuminemia. Pleural effusions or ascites may be detected on clinical examination when large, but the demonstration of fluid on ultrasound may assist in the early identification of dengue shock syndrome. The use of ultrasound is limited by lack of availability in many settings.

Classification

The World Health Organization's 2009 classification divides dengue fever into two groups: uncomplicated and severe. This replaces the 1997 WHO classification, which was simplified as it was found to be too restrictive, but the older classification is still widely used. The 1997 classification divided dengue into undifferentiated fever, dengue fever, and dengue hemorrhagic fever. Dengue hemorrhagic fever was subdivided further into four grades (grade I–IV). Grade I is the presence only of easy bruising or a positive "tourniquet test" in someone with fever, grade II is the presence of spontaneous bleeding into the skin and elsewhere, grade III is the clinical evidence of shock, and grade IV is shock so severe that blood pressure and pulse cannot be detected. Grades III and IV are referred to as "dengue shock syndrome".

Virology and serology

Dengue fever may also be diagnosed by microbiological laboratory testing. This can be done by virus isolation in cell cultures, nucleic acid detection by PCR, viral antigen detection or specific antibodies (serology). Virus isolation and nucleic acid detection are more accurate than antigen detection, but these tests are not widely available due to their greater cost. All tests may be negative in the early stages of the disease.

Apart from serology, laboratory tests are only of diagnostic value during the acute phase of the illness. Tests for dengue virus-specific antibodies, types IgG and IgM, can be useful in confirming a diagnosis in the later stages of the infection. Both IgG and IgM are produced after 5–7 days. The highest levels (titres) of IgM are detected following a primary infection, but IgM is also produced in secondary and tertiary infections. The IgM becomes undetectable 30–90 days after a primary infection, but earlier following re-infections. IgG, by contrast, remains detectable for over 60 years and, in the absence of symptoms, is a useful indicator of past infection. After a primary infection the IgG

reaches peak levels in the blood after 14–21 days. In subsequent re-infections levels peak earlier and the titres are usually higher. Both IgG and IgM provide protective immunity to the infecting serotype of the virus. In the laboratory test the IgG and the IgM antibodies can cross-react with other flaviviruses, such as yellow fever virus, which can make the interpretation of the serology difficult. The detection of IgG alone is not considered diagnostic unless blood samples are collected 14 days apart and a greater than fourfold increase in levels of specific IgG is detected. In a person with symptoms, the detection of IgM is considered diagnostic.

Prevention

A 1920s photograph of efforts to disperse standing water and thus decrease mosquito populations.

There are currently no approved vaccines for the dengue virus. Prevention thus depends on control of and protection from the bites of the mosquito that transmits it. The World Health Organization recommends a Integrated Vector Control program consisting of five elements: (1) Advocacy, social mobilization and legislation to ensure that public health bodies and communities are strengthened, (2) collaboration between the health and other

sectors (public and private), (3) an integrated approach to disease control to maximize use of resources, (4) evidence-based decision making to ensure any interventions are targeted appropriately and (5) capacity-building to ensure an adequate response to the local situation.

The primary method of controlling *A. aegypti* is by eliminating its habitats. This may be done by emptying containers of water or by adding insecticides or biological control agents to these areas. Reducing open collections of water through environmental modification is the preferred method of control, given the concerns of negative health effect from insecticides and greater logistical difficulties with control agents. People may prevent mosquito bites by wearing clothing that fully covers the skin and/or the application of insect repellent (DEET being the most effective).

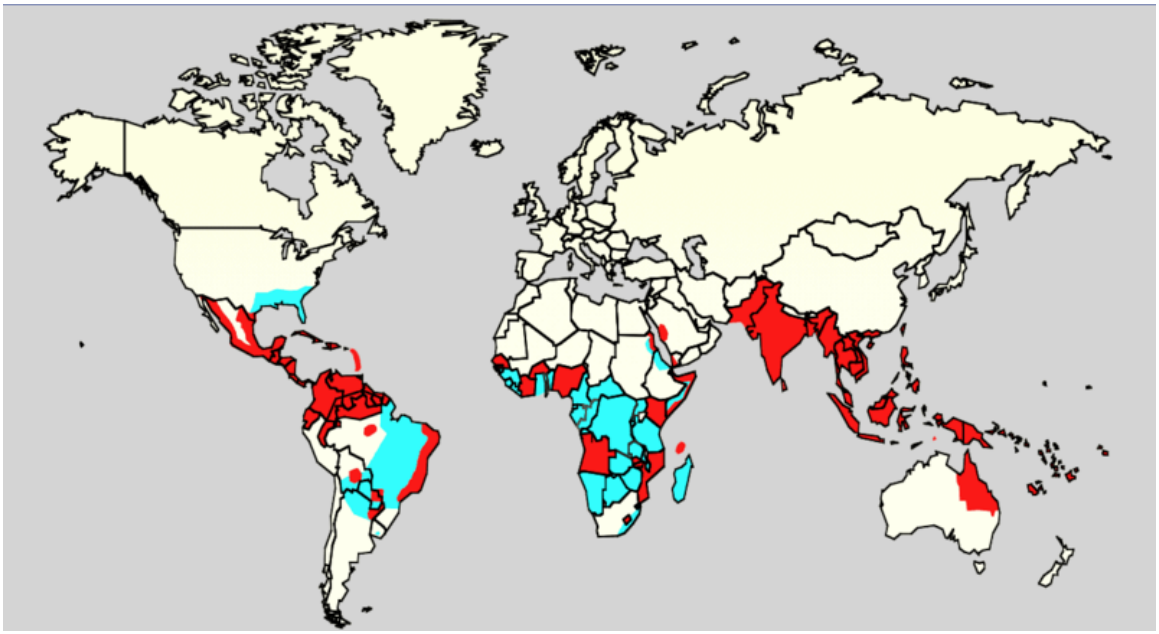
Management

There are no specific treatments for the dengue fever virus. Treatment depends on the symptoms, and may vary from advice to drink plenty of fluids such as oral rehydration solution at home with close follow up, to admission to hospital for carefully titrated isotonic intravenous fluids and/or blood transfusions. A decision for hospital admission is typically based on the presence or absence of the "warning signs" listed above, and the presence of preexisting health conditions.

Intravenous fluids if used are usually only needed for one or two days. Fluids are titrated to a urinary output of 0.5–1 mL/kg/hr, stable vital signs and normalization of hematocrit. Procedures that increase bleeding risk such as nasogastric tubes, intramuscular injections and arterial punctures are avoided. Acetaminophen may be used for fever and discomfort while NSAIDs such as ibuprofen or aspirin are avoided due to an increased bleeding risk. The need for blood transfusions is based on the presence of unstable vital signs and a decreasing haematocrit rather than the usual haematocrit of less than 30% used in sepsis. Packed red blood cells or whole blood are recommended, while platelets and fresh frozen plasma are usually not.

During the recovery phase intravenous fluids are discontinued to prevent a state of fluid overload. If fluid overload occurs and vital signs are stable, stopping further fluid may be all that is needed. If a person is outside of the critical phase, the loop diuretic furosemide may be used to eliminate excess fluid from the circulation.

Epidemiology



Dengue distribution in 2006.
Red: Epidemic dengue and *Ae. aegypti*
Aqua: Just *Ae. aegypti*.

Most people with dengue recover without any ongoing problems. The mortality is 1–5% without treatment, and less than 1% with adequate treatment. Severe disease carries a mortality of 26%. Dengue is believed to infect 50 to 100 million people worldwide a year with 1/2 million life-threatening infections requiring hospitalization, resulting in approximately 12,500-25,000 deaths.

The burden of disease from dengue is estimated to be similar to other childhood and tropical diseases, such as tuberculosis, at 1600 disability-adjusted life years per million population. It is the most common viral disease transmitted by arthropods. As a tropical disease it is deemed only second in importance to malaria. While once exclusively a tropical disease it has become global, and is endemic in more than 110 countries. The World Health Organization counts dengue as one of sixteen neglected tropical diseases.

The incidence of dengue increased 30 fold between 1960 and 2010. This increase is believed to be due to a combination of urbanization, population growth, increased international travel, and global warming. The geographical distribution is around the equator with 70% of the total 2.5 billion people living in endemic areas from Asia and the Pacific. In the United States, the rate of dengue infection among those who return from an endemic area with a fever is 2.9–8.0%, and it is the second most common infection after malaria to be diagnosed in this group.

Until 2003, dengue was classified as a potential bioterrorism agent, but subsequent reports removed this classification as it was deemed too difficult to transfer and only caused hemorrhagic fever in a relatively small proportion of people.

History

Etymology

The origins of the word "dengue" are not clear, but one theory is that it is derived from the Swahili phrase *Ka-dinga pepo*, which describes the disease as being caused by an evil spirit. The Swahili word *dinga* may possibly have its origin in the Spanish word *dengue* meaning fastidious or careful, which would describe the gait of a person suffering the bone pain of dengue fever. Alternatively, the use of the Spanish word may derive from the similar-sounding Swahili. Slaves in the West Indies who contracted dengue were said to have the posture and gait of a dandy, and the disease was known as "dandy fever".

The term "break-bone fever" was first applied by physician and Founding Father Benjamin Rush, in a 1789 report of the 1780 epidemic in Philadelphia. In the report he uses primarily the more formal term "bilious remitting fever". The term dengue fever only came into general use after 1828. Other historical terms include "breakheart fever" and "la dengue". Terms for severe disease include: "infectious thrombocytopenic purpura" and "Philippine", "Thai", or "Singapore hemorrhagic fever".

Discovery

The first record of a case of probable dengue fever is in a Chinese medical encyclopedia from the Jin Dynasty (265–420 AD) which referred to a "water poison" associated with flying insects. There have been descriptions of epidemics in the 17th century, but the most plausible early reports of dengue epidemics are from 1779 and 1780, when an epidemic swept Asia, Africa and North America. From that time until 1940, epidemics were infrequent.

In 1906, transmission by the *Aedes* mosquitoes was confirmed, and in 1907 dengue was the second disease (after yellow fever) that was shown to be caused by a virus. Further investigations by John Burton Cleland and Joseph Franklin Siler completed the basic understanding of dengue transmission.

The marked rise of spread of dengue during and after the Second World War has been attributed to ecologic disruption. The same trends also led to the spread of different serotypes of the disease to different areas, and the emergence of dengue hemorrhagic fever, which was first reported in the Philippines in 1953. In the 1970s, it became a major cause of child mortality. Around the same time it emerged in the Pacific and the Americas. Dengue hemorrhagic fever and dengue shock syndrome were first noted in Middle and Southern America in 1981, as DENV-2 was contracted by people who had previously been infected with DENV-1 several years earlier.

Research directions

Current research efforts to prevent and treat dengue have included different means of vector control, vaccine development, and antiviral drugs.

With regards to vector control, a number of novel methods have been used to reduce mosquito numbers with some success including the placement of the fish *Poecilia reticulata* or copepods in standing water to eat the mosquito larva.

There are ongoing programs working on a dengue vaccine to cover all four serotypes. One of the concerns is that a vaccine may increase the risk of severe disease through antibody-dependent enhancement. The ideal vaccine is safe, effective after one or two injections, covers all serotypes, does not contribute to ADE, is easily transported and stored, and is both affordable and cost-effective. A number of vaccines are currently undergoing testing. It is hoped that the first products will be commercially available by 2015.

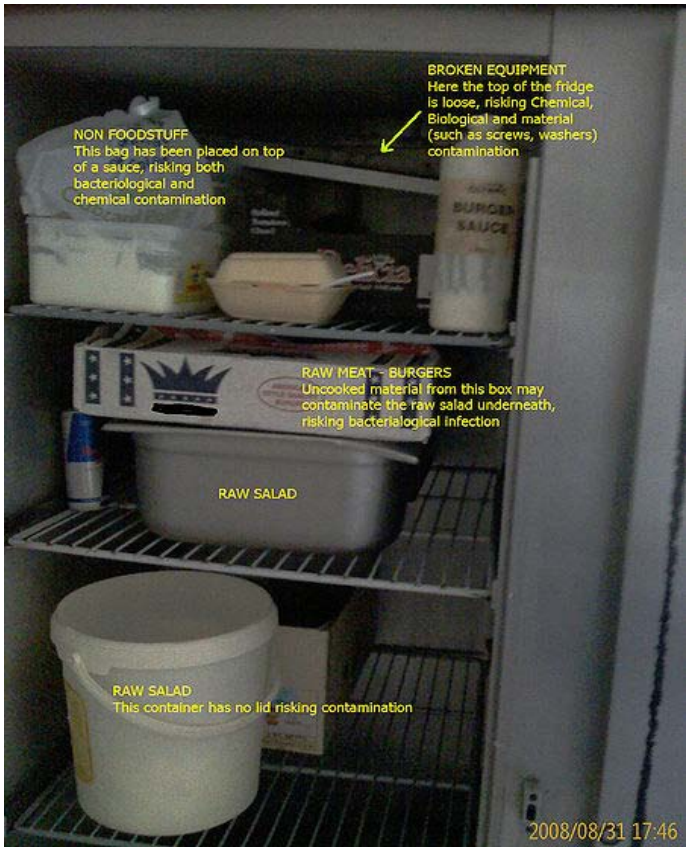
Apart from attempts to control the spread of the *Aedes* mosquito and work to develop a vaccine against dengue, there are ongoing efforts to develop antiviral drugs that might be used to treat attacks of dengue fever and prevent severe complications. Discovery of the structure of the viral proteins may aid the development of effective drugs. There are several plausible targets. The first approach is inhibition of the viral RNA-dependent RNA polymerase (coded by NS5), which copies the viral genetic material, with nucleoside analogs. Secondly, it may be possible to develop specific inhibitors of the viral protease (coded by NS3), which splices viral proteins. Finally, it may be possible to develop entry inhibitors, which stop the virus entering cells, or inhibitors of the 5' capping process, which is required for viral replication.

Chapter 9

Foodborne Illness

Foodborne illness (also **foodborne disease** and colloquially referred to as **food poisoning**) is any illness resulting from the consumption of contaminated food, pathogenic bacteria, viruses, or parasites that contaminate food, rather than chemical or natural toxins.

Causes



Poorly stored food in a refrigerator

Foodborne illness usually arises from improper handling, preparation, or food storage. Good hygiene practices before, during, and after food preparation can reduce the chances

of contracting an illness. There is a consensus in the public health community that regular hand-washing is one of the most effective defenses against the spread of foodborne illness. The action of monitoring food to ensure that it will not cause foodborne illness is known as **food safety**. Foodborne disease can also be caused by a large variety of toxins that affect the environment.

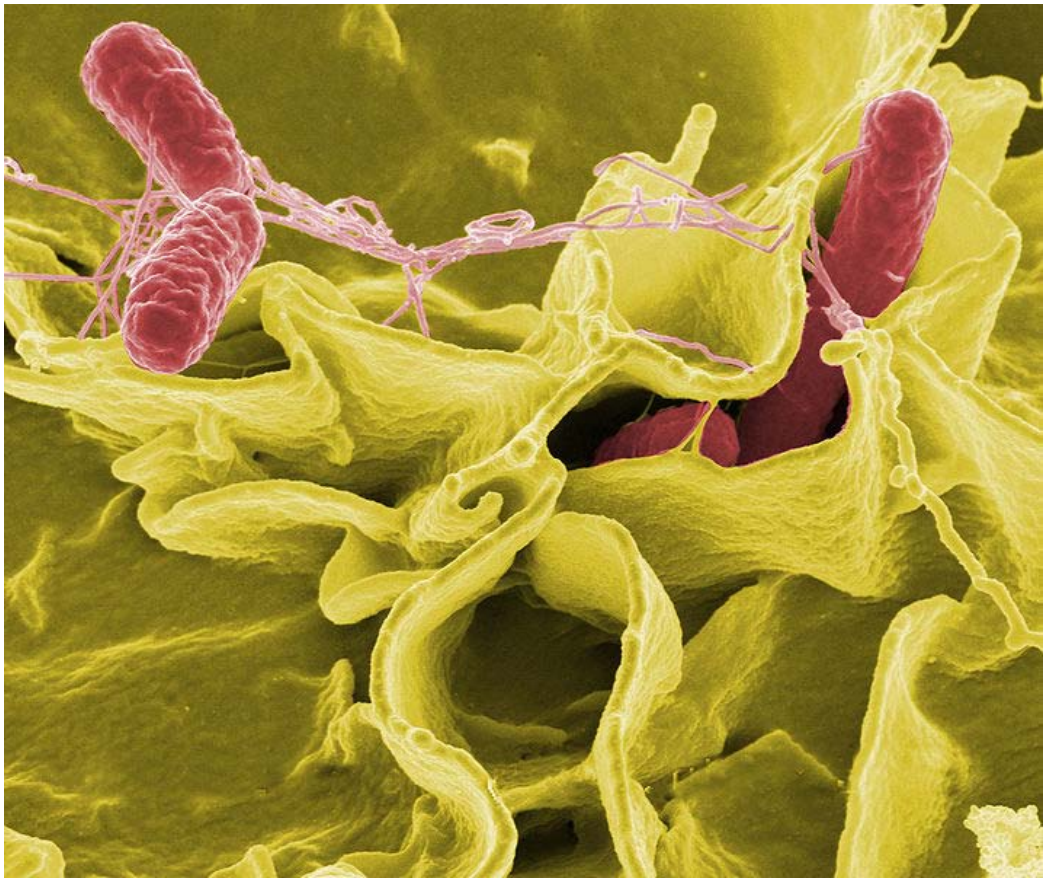
Foodborne illness can also be caused by pesticides or medicines in food and naturally toxic substances like poisonous mushrooms or reef fish.

Bacteria

Bacteria are a common cause of foodborne illness. In the United Kingdom during 2000 the individual bacteria involved were as follows: *Campylobacter jejuni* 77.3%, *Salmonella* 20.9%, *Escherichia coli O157:H7* 1.4%, and all others less than 0.1%. In the past, bacterial infections were thought to be more prevalent because few places had the capability to test for norovirus and no active surveillance was being done for this particular agent. Symptoms for bacterial infections are delayed because the bacteria need time to multiply. They are usually not seen until 12–72 hours or more after eating contaminated food.

Most common bacterial foodborne pathogens are:

- *Campylobacter jejuni* which can lead to secondary Guillain–Barré syndrome and periodontitis
- *Clostridium perfringens*, the "cafeteria germ"
- *Salmonella* spp. – its *S. typhimurium* infection is caused by consumption of eggs or poultry that are not adequately cooked or by other interactive human-animal pathogens



Salmonella

- *Escherichia coli O157:H7* enterohemorrhagic (EHEC) which causes hemolytic-uremic syndrome

Other **common** bacterial foodborne pathogens are:

- *Bacillus cereus*
- *Escherichia coli*, other virulence properties, such as enteroinvasive (EIEC), enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroaggregative (EAEC or EAgEC)
- *Listeria monocytogenes*
- *Shigella* spp.
- *Staphylococcus aureus*
- *Streptococcus*
- *Vibrio cholerae*, including O1 and non-O1
- *Vibrio parahaemolyticus*
- *Vibrio vulnificus*
- *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*

Less common bacterial agents:

- *Brucella* spp.
- *Corynebacterium ulcerans*
- *Coxiella burnetii* or Q fever
- *Plesiomonas shigelloides*

Exotoxins

In addition to disease caused by direct bacterial infection, some foodborne illnesses are caused by exotoxins which are excreted by the cell as the bacterium grows. Exotoxins can produce illness even when the microbes that produced them have been killed. Symptoms typically appear after 24 hours depending on the amount of toxin ingested.

- *Clostridium botulinum*
- *Clostridium perfringens*
- *Staphylococcus aureus*
- *Bacillus cereus*

For example *Staphylococcus aureus* produces a toxin that causes intense vomiting. The rare but potentially deadly disease botulism occurs when the anaerobic bacterium *Clostridium botulinum* grows in improperly canned low-acid foods and produces botulin, a powerful paralytic toxin.

Pseudoalteromonas tetraodonis, certain species of Pseudomonas and Vibrio, and some other bacteria, produce the lethal tetrodotoxin, which is present in the tissues of some living animal species rather than being a product of decomposition.

Mycotoxins and alimentary mycotoxicoses

The term **alimentary mycotoxicoses** refers to the effect of poisoning by Mycotoxins through food consumption. Mycotoxins sometimes have important effects on human and animal health. For example, an outbreak which occurred in the UK in 1960 caused the death of 100,000 turkeys which had consumed aflatoxin-contaminated peanut meal. In the USSR in World War II, 5000 people died due to Alimentary Toxic Aleukia (ALA). The common foodborne Mycotoxins include:

- Aflatoxins – originated from Aspergillus parasiticus and Aspergillus flavus. They are frequently found in tree nuts, peanuts, maize, sorghum and other oilseeds, including corn and cottonseeds. The pronounced forms of Aflatoxins are those of B1, B2, G1, and G2, amongst which Aflatoxin B1 predominantly targets the liver, which will result in necrosis, cirrhosis, and carcinoma. In the US, the acceptable level of total aflatoxins in foods is less than 20 μg/kg, except for Aflatoxin M1 in milk, which should be less than 0.5 μg/kg.
- Altertoxins – are those of Alternariol (AOH), Alternariol methyl ether (AME), Altenuene (ALT), Altertoxin-1 (ATX-1), Tenuazonic acid (TeA) and Radicinin

(RAD), originated from Alternaria spp. Some of the toxins can be present in sorghum, ragi, wheat and tomatoes. Some research has shown that the toxins can be easily cross-contaminated between grain commodities, suggesting that manufacturing and storage of grain commodities is a critical practice.

- Citrinin
- Citreoviridin
- Cyclopiazonic acid
- Cytochalasins
- Ergot alkaloids / Ergopeptine alkaloids – Ergotamine
- Fumonisins – Crop corn can be easily contaminated by the fungi Fusarium moniliforme, and its Fumonisin B1 will cause Leukoencephalomalacia (LEM) in horses, Pulmonary edema syndrome (PES) in pigs, liver cancer in rats and Esophageal cancer in humans. For human and animal health, both the FDA and the EC have regulated the content levels of toxins in food and animal feed.
- Fusaric acid
- Fusarochromanone
- Kojic acid
- Lolitrem alkaloids
- Moniliformin
- 3-Nitropropionic acid
- Nivalenol
- Ochratoxins – In Australia, The Limit of Reporting (LOR) level for Ochratoxin A (OTA) analyses in 20th Australian Total Diet Survey was 1 μg/kg, whereas the EC restricts the content of OTA to 5 μg/kg in cereal commodities, 3 μg/kg in processed products and 10 μg/kg in dried vine fruits.
- Oosporeine
- Patulin – Currently, this toxin has been advisably regulated on fruit products. The EC and the FDA have limited it to under 50 μg/kg for fruit juice and fruit nectar, while limits of 25 μg/kg for solid-contained fruit products and 10 μg/kg for baby foods were specified by the EC.
- Phomopsins
- Sporidesmin A
- Sterigmatocystin
- Tremorgenic mycotoxins – Five of them have been reported to be associated with molds found in fermented meats. These are Fumitremorgen B, Paxilline, Penitrem A, Verrucosidin, and Verruculogen.
- Trichothecenes – sourced from Cephalosporium, Fusarium, Myrothecium, Stachybotrys and Trichoderma. The toxins are usually found in molded maize, wheat, corn, peanuts and rice, or animal feed of hay and straw. Four trichothecenes, T-2 toxin, HT-2 toxin, diacetoxyscirpenol (DAS) and deoxynivalenol (DON) have been most commonly encountered by humans and animals. The consequences of oral intake of, or dermal exposure to, the toxins will result in Alimentary toxic aleukia, neutropenia, aplastic anemia, thrombocytopenia and/or skin irritation. In 1993, the FDA issued a document for the content limits of DON in food and animal feed at an advisory level. In 2003,

US published a patent that is very promising for farmers to produce a trichothecene-resistant crop.
- Zearalenone
- Zearalenols

Emerging foodborne pathogens

Many foodborne illnesses remain poorly understood. Approximately sixty percent of outbreaks are caused by unknown sources.

- *Aeromonas hydrophila*, *Aeromonas caviae*, *Aeromonas sobria*

Preventing bacterial food poisoning

Proper storage and refrigeration of food help in the prevention of food poisoning

Prevention is mainly the role of the state, through the definition of strict rules of hygiene and a public services of veterinary surveying of animal products in the food chain, from farming to the transformation industry and delivery (shops and restaurants). This regulation includes:

- traceability: in a final product, it must be possible to know the origin of the ingredients (originating farm, identification of the harvesting or of the animal) and where and when it was processed; the origin of the illness can thus be tracked and solved (and possibly penalized), and the final products can be removed from the sale if a problem is detected;
- enforcement of hygiene procedures like HACCP and the "cold chain";
- power of control and of law enforcement of veterinarians.

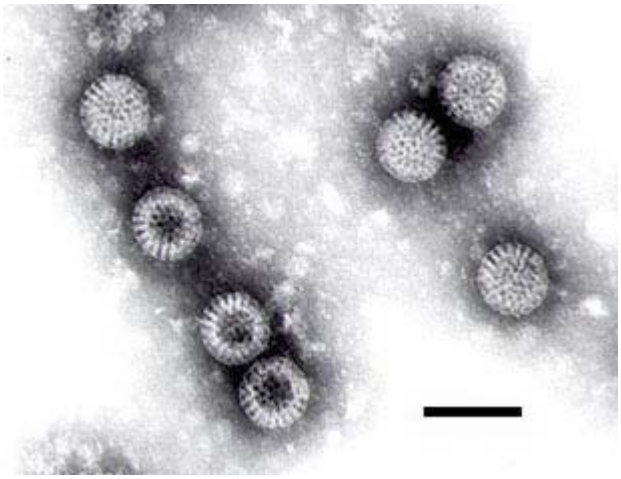
In August 2006, the United States Food and Drug Administration approved Phage therapy which involves spraying meat with viruses that infect bacteria, and thus preventing infection. This has raised concerns, because without mandatory labelling consumers would not be aware that meat and poultry products have been treated with the spray.

At home, prevention mainly consists of good food safety practices. Many forms of bacterial poisoning can be prevented even if food is contaminated by cooking it sufficiently, and either eating it quickly or refrigerating it effectively. Many toxins, however, are not destroyed by heat treatment.

Viruses

Viral infections make up perhaps one third of cases of food poisoning in developed countries. In the US, more than 50% of cases are viral and noroviruses are the most common foodborne illness, causing 57% of outbreaks in 2004. Foodborne viral infection are usually of intermediate (1–3 days) incubation period, causing illnesses which are self-limited in otherwise healthy individuals, and are similar to the bacterial forms described above.

- Enterovirus
- Hepatitis A is distinguished from other viral causes by its prolonged (2–6 week) incubation period and its ability to spread beyond the stomach and intestines, into the liver. It often induces jaundice, or yellowing of the skin, and rarely leads to chronic liver dysfunction. The virus has been found to cause the infection due to the consumption of fresh-cut produce which has fecal contamination.
- Hepatitis E
- Norovirus
- Rotavirus



Rotavirus

Parasites

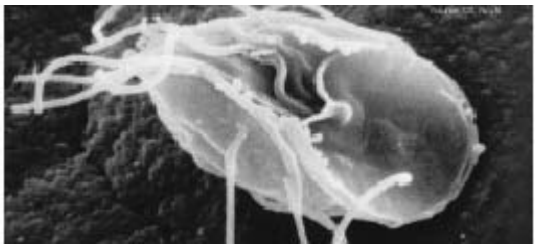
Most foodborne parasites are zoonoses.

- Platyhelminthes:
  - *Diphyllobothrium* sp.
  - *Nanophyetus* sp.
  - *Taenia saginata*
  - *Taenia solium*

The scolex of *Taenia solium*

- *Fasciola hepatica*

- Nematode:
  - *Anisakis* sp.
  - *Ascaris lumbricoides*
  - *Eustrongylides* sp.
  - *Trichinella spiralis*
  - *Trichuris trichiura*
- Protozoa:
  - *Acanthamoeba* and other free-living amoebae
  - *Cryptosporidium parvum*
  - *Cyclospora cayetanensis*
  - *Entamoeba histolytica*
  - *Giardia lamblia*



Giardia lamblia

  - *Sarcocystis hominis*
  - *Sarcocystis suihominis*
  - *Toxoplasma gondii*

Natural toxins

Several foods can naturally contain toxins, many of which are not produced by bacteria. Plants in particular may be toxic; animals which are naturally poisonous to eat are rare. In evolutionary terms, animals can escape being eaten by fleeing; plants can use only passive defenses such as poisons and distasteful substances, for example capsaicin in chili peppers and pungent sulfur compounds in garlic and onions. Most animal poisons are not synthesised by the animal, but acquired by eating poisonous plants to which the animal is immune, or by bacterial action.

- Alkaloids
- Ciguatera poisoning
- Grayanotoxin (honey intoxication)
- Mushroom toxins
- Phytohaemagglutinin (red kidney bean poisoning; destroyed by boiling)
- Pyrrolizidine alkaloids

- Shellfish toxin, including paralytic shellfish poisoning, diarrhetic shellfish poisoning, neurotoxic shellfish poisoning, amnesic shellfish poisoning and ciguatera fish poisoning
- Scombrotoxin
- Tetrodotoxin (fugu fish poisoning)

Some plants contain substances which are toxic in large doses, but have therapeutic properties in appropriate dosages.

- Foxglove contains cardiac glycosides.
- Poisonous hemlock (conium) has medicinal uses.

Other pathogenic agents

- Prions, resulting in Creutzfeldt-Jakob disease

"Ptomaine poisoning"

An early theory on the causes of food poisoning involved *ptomaines* (from Greek *ptōma*, "fall, fallen body, corpse"), alkaloids found in decaying animal and vegetable matter. While some alkaloids do cause poisoning, the discovery of bacteria left the ptomaine theory obsolete and the word *ptomaine* is no longer used scientifically.

Mechanism

Incubation period

The delay between consumption of a contaminated food and appearance of the first symptoms of illness is called the incubation period. This ranges from hours to days (and rarely months or even years, such as in the case of Listeriosis or Creutzfeldt-Jacob disease), depending on the agent, and on how much was consumed. If symptoms occur within 1–6 hours after eating the food, it suggests that it is caused by a bacterial toxin or a chemical rather than live bacteria.

The long incubation period of many foodborne illnesses tends to cause sufferers to attribute their symptoms to "stomach flu".

During the incubation period, microbes pass through the stomach into the intestine, attach to the cells lining the intestinal walls, and begin to multiply there. Some types of microbes stay in the intestine, some produce a toxin that is absorbed into the bloodstream, and some can directly invade the deeper body tissues. The symptoms produced depend on the type of microbe.

Infectious dose

The infectious dose is the amount of agent that must be consumed to give rise to symptoms of foodborne illness, and varies according to the agent and the consumer's age and overall health. In the case of *Salmonella* a relatively large inoculum of 1 million to 1 billion organisms is necessary to produce symptoms in healthy human volunteers , as *Salmonellae* are very sensitive to acid. An unusually high stomach pH level (low acidity) greatly reduces the number of bacteria required to cause symptoms by a factor of between 10 and 100.

Epidemiology

Every year there are an estimated 76 million foodborne illnesses in the United States (26,000 cases for 100,000 inhabitants), 2 million in the United Kingdom (3,400 cases for 100,000 inhabitants) and 750,000 in France (1,220 cases for 100,000 inhabitants).

United States

In the United States, using FoodNet data from 1996–1998, the CDCP estimated there were 76 million foodborne illnesses (26,000 cases for 100,000 inhabitants):

- 325,000 were hospitalized (111 per 100,000 inhabitants);
- 5,000 people died (1.7 per 100,000 inhabitants.).
- Major pathogens from foodborne illness in the United States cost upwards of US $35 billion in medical costs and lost productivity (1997)

Causes of foodborne illness in U. S.

	Cause	Annual cases	Rate (per 100,000 inhabitants)
1	*Norwalk-like viruses*	9,200,000 cases	X
2	*Salmonella*	1,341,873 cases	X
3	Campylobacter	1,963,141 cases	X
4	*Toxoplasma gondii*	112,500 cases	X
5	Listeria monocytogenes	2,493 cases	X
	Total	60854 cases	X

Causes of death by foodborne illness in U. S.

	Cause	Annual deaths	Rate (per 100,000 inhabitants)
1	*Salmonella*	553 cases	0.21
2	*Listeria*	499 cases	0.19
3	toxoplasma	374 cases	0.14
4	*Norwalk-like viruses*	124 cases	0.046
5	Campylobacter	99 cases	0.037
	Gastrointestinal illness, unknown eitiology	5100 cases	1.9

France

In France, for 750,000 cases(1,210 per 100,000 inhabitants):

- 70,000 people consulted in the emergency department of an hospital (113 per 100,000 inhabitants.);
- 113,000 people were hospitalized (24 per 100,000 inhabitants);
- 400 people died (0.9 per 100,000 inhabitants).

Causes of foodborne illness in France

Cause	Annual cases	Rate (per 100,000 inhabitants)
1 *Salmonella*	~8,000 cases	13
2 *Campylobacter*	~3,000 cases	4.8
3 Parasites incl. Toxoplasma	~500 cases ~400 cases	0.8 0.65
4 *Listeria*	~300 cases	0.5
5 Hepatitis A	~60 cases	0.1

Causes of death by foodborne illness in France

Cause	Annual	Rate (per 100,000 inhabitants)
1 *Salmonella*	~300 cases	0.5
2 *Listeria*	~80 cases	0.13
3 Parasites	~37 cases	0.06 (95% due to toxoplasma)
4 *Campylobacter*	~15 cases	0.02
5 Hepatitis A	~2 cases	0.003

Australia

In Australia, there are an estimated 5.4 million cases of food-borne illness every year, causing:

- 18,000 hospitalizations
- 120 deaths
- 2.1 million lost days off work
- 1.2 million doctor consultations
- 300,000 prescriptions for antibiotics

Outbreaks

The vast majority of reported cases of foodborne illness occur as individual or sporadic cases. The origin of most sporadic cases is undetermined. In the United States, where people eat outside the home frequently, most outbreaks (58%) originate from commercial food facilities (2004 FoodNet data). An outbreak is defined as occurring when two or more people experience similar illness after consuming food from a common source.

Often, a combination of events contributes to an outbreak, for example, food might be left at room temperature for many hours, allowing bacteria to multiply which is compounded by inadequate cooking which results in a failure to kill the dangerously elevated bacterial levels.

Outbreaks are usually identified when those affected know each other. However, more and more, outbreaks are identified by public health staff from unexpected increases in laboratory results for certain strains of bacteria. Outbreak detection and investigation in the United States is primarily handled by local health jurisdictions and is inconsistent from district to district. It is estimated that 1–2% of outbreaks are detected.

Society and culture

Global impact

In modern times, rapid globalization of food production and trade has increased the potential likelihood of food contamination. Many outbreaks of foodborne diseases that were once contained within a small community may now take place on global dimensions. Food safety authorities all over the world have acknowledged that ensuring food safety must not only be tackled at the national level but also through closer linkages among food safety authorities at the international level. This is important for exchanging routine information on food safety issues and to have rapid access to information in case of food safety emergencies."

It is difficult to estimate the global incidence of foodborne disease, but it has been reported that in the year 2000 about 2.1 million people died from diarrhoeal diseases. Many of these cases have been attributed to contamination of food and drinking water. Additionally, diarrhoea is a major cause of malnutrition in infants and young children.

Even in industrialized countries, up to 30% of the population of people have been reported to suffer from foodborne diseases every year. In the U.S, around 76 million cases of foodborne diseases, which resulted in 325,000 hospitalizations and 5,000 deaths, are estimated to occur each year. Developing countries in particular are worst affected by foodborne illnesses due to the presence of a wide range of diseases, including those caused by parasites. Foodborne illnesses can and did inflict serious and extensive harm on society. In 1994, an outbreak of salmonellosis due to contaminated ice cream occurred in the USA, affecting an estimated 224,000 persons. In 1988, an outbreak of hepatitis A, resulting from the consumption of contaminated clams, affected some 300,000 individuals in China.

Food contamination creates an enormous social and economic strain on societies. In the U.S., diseases caused by the major pathogens alone are estimated to cost up to US $35 billion annually (1997) in medical costs and lost productivity. The re-emergence of cholera in Peru in 1991 resulted in the loss of US $500 million in fish and fishery product exports that year.

United Kingdom

In postwar Aberdeen (1964) a large scale (>400 cases) outbreak of Typhoid occurred, this was caused by contaminated corned beef which had been imported from Argentina The corned beef was placed in cans and because the cooling plant had failed, cold river water from the Plate estuary was used to cool the cans. One of the cans had a defect and the meat inside was contaminated. This meat was then sliced using a meat slicer in a shop in Aberdeen, and a lack of cleaning the machinery lead to spreading the contamination to other meats cut in the slicer. These meats were then eaten by the people of Aberdeen who then became ill.

In the UK serious outbreaks of food-borne illness since the 1970s prompted key changes in UK food safety law. These included the death of 19 patients in the Stanley Royd Hospital outbreak and the bovine spongiform encephalopathy (BSE, mad cow disease) outbreak identified in the 1980s. The death of 17 people in the 1996 Wishaw outbreak of E. coli O157 was a precursor to the establishment of the Food Standards Agency which, according to Tony Blair in the 1998 white paper *A Force for Change* Cm 3830 "would be powerful, open and dedicated to the interests of consumers".

United States

In 1999 an estimated 5,000 deaths, 325,000 hospitalizations and 76 million illnesses were foodborne in the US.

In 2001, the Center for Science in the Public Interest petitioned the United States Department of Agriculture to require meat packers to remove spinal cords before processing cattle carcasses for human consumption, a measure designed to lessen the risk of infection by variant Creutzfeldt-Jakob disease. The petition was supported by the American Public Health Association, the Consumer Federation of America, the Government Accountability Project, the National Consumers League, and Safe Tables Our Priority. This was opposed by the National Cattlemen's Beef Association, the National Renderers Association, the National Meat Association, the Pork Producers Council, sheep raisers, milk producers, the Turkey Federation, and eight other organizations from the animal-derived food industry. This was part of a larger controversy regarding the United States' violation of World Health Organization proscriptions to lessen the risk of infection by variant Creutzfeldt-Jakob disease.

None of the US Department of Health and Human Services targets regarding incidence of foodborne infections were reached in 2007.

Organizations

World Health Organization Food Safety Department

> The WHO provides scientific advice for organizations and the public on issues concerning the safety of food. It serves as a medium linking the food safety systems in countries around the world. Food safety is currently one of WHO's top

ten priorities. Food Safety is one of the major issues in our world today, and the Organization calls for more systematic and aggressive steps to be taken to significantly reduce the risk of foodborne diseases.

The Department of Food Safety, Zoonoses and Foodborne Diseases

The Department of Food Safety, Zoonoses and Foodborne Diseases is a department under the WHO. Its mission is to reduce the serious negative impact of foodborne diseases worldwide. According to the WHO website, food and waterborne diarrhoeal diseases are leading causes of illness and death in less developed countries, killing approximately 3.8 million people annually, most of whom are children.

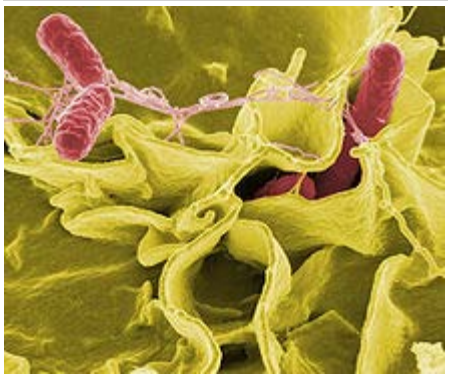
WHO works closely with the Food and Agriculture Organization of the United Nations (FAO) to address food safety issues along the entire food production chain--from production to consumption--using new methods of risk analysis. These methods provide efficient, science-based tools to improve food safety, thereby benefiting both public health and economic development.

The International Food Safety Authorities Network (INFOSAN)

This network is intended to complement and support the existing WHO Global Outbreak Alert and Response Network (GOARN) which includes a Chemical Alert and Response component.

Chapter 10

Salmonella

Salmonella

Scientific classification
Kingdom: Bacteria
Class: Gammaproteobacteria
Order: Enterobacteriales
Family: Enterobacteriaceae
Genus: ***Salmonella*** Lignieres 1900
Species
S. bongori *S. enterica*

Salmonella is a genus of rod-shaped, Gram-negative, non-spore-forming, predominantly motile enterobacteria with diameters around 0.7 to 1.5 µm, lengths from 2 to 5 µm, and flagella which grade in all directions (i.e. peritrichous). They are chemoorganotrophs, obtaining their energy from oxidation and reduction reactions using organic sources, and are facultative anaerobes. Most species produce hydrogen sulfide, which can readily be detected by growing them on media containing ferrous sulfate, such as TSI. Most isolates exist in two phases: a motile phase I and a nonmotile phase II. Cultures that are nonmotile upon primary culture may be switched to the motile phase using a Cragie tube.

Salmonella is closely related to the *Escherichia* genus and are found worldwide in cold- and warm-blooded animals (including humans), and in the environment. They cause illnesses like typhoid fever, paratyphoid fever, and the foodborne illness.

Salmonella is typically voicing the initial letter "L," although it is named for pathologist Daniel Elmer Salmon.

Salmonella as disease-causing agents

Salmonella infections are zoonotic and can be transferred between humans and nonhuman animals. Many infections are due to ingestion of contaminated food. A distinction is made between enteritis *Salmonella* and typhoid/paratyphoid *Salmonella*, where the latter — because of a special virulence factor and a capsule protein (virulence antigen) — can cause serious illness, such as *Salmonella enterica subsp. enterica* serovar Typhi. *Salmonella* typhi. is adapted to humans and does not occur in animals.

Enteritis *Salmonelliosis* or Food Poisoning *Salmonella*

This is a group consisting of potentially all other serotypes (over a thousand) of the *Salmonella* bacterium, most of which have never been found in humans. These are encountered in various *Salmonella* species, most having never been linked to a specific host, and can also infect humans. It is therefore a zoonotic disease. The organism enters through the digestive tract and must be ingested in large numbers to cause disease in healthy adults. Gastric acidity is responsible for the destruction of the majority of ingested bacteria. The infection usually occurs as a result of massive ingestion of foods in which the bacteria are highly concentrated similarly to a culture medium. However, infants and young children are much more susceptible to infection, easily achieved by ingesting a small number of bacteria. It has been shown that, in infants, the contamination could be through inhalation of bacteria-laden dust. After a short incubation period of a few hours to one day, the germ multiplies in the intestinal lumen causing an intestinal inflammation with diarrhoea that is often muco-purulent and bloody. In infants, dehydration can cause a state of severe toxicosis. The symptoms are usually mild. There is normally no sepsis, but it can occur exceptionally as a complication in weakened elderly patients (Hodgkin's disease, eg.). Extraintestinal localizations are possible, especially *Salmonella* meningitis in children, osteitis, etc. Enteritis *Salmonella* (e.g., *Salmonella enterica* subsp. *enterica* serovar *enteritidis*) can cause diarrhoea, which usually does not require antibiotic treatment. However, in people at risk such as infants, small children, the elderly, *Salmonella* infections can become very serious, leading to complications. If these are not treated, HIV patients and those with suppressed immunity can become seriously ill. Children with sickle cell anaemia who are infected with *Salmonella* may develop osteomyelitis.

In Germany, *Salmonella* infections must be reported. Between 1990 and 2005, the number of officially recorded cases decreased from approximately 200,000 cases to approximately 50,000. It is estimated that every fifth person in Germany is a carrier of

Salmonella. In the USA, there are approximately 40,000 cases of *Salmonella* infection reported each year. According to the World Health Organization, over 16 million people worldwide are infected with typhoid fever each year, with 500,000 to 600,000 fatal cases.

Salmonella can survive for weeks outside a living body. They have been found in dried excrement after more than 2.5 years. *Salmonella* are not destroyed by freezing. Ultraviolet radiation and heat accelerate their demise; they perish after being heated to 55 °C (131 °F) for one hour, or to 60 °C (140 °F) for half an hour. To protect against *Salmonella* infection, it is recommended that food be heated for at least ten minutes at 75 °C (167 °F) so that the centre of the food reaches this temperature.

The AvrA toxin injected by the type three secretion system of *Salmonella typhimurium* works to inhibit the innate immune system by virtue of its serine/threonine acetyltransferase activity and requires binding to eukaryotic target cell phytic acid (IP6). This leaves the host more susceptible to infection. In a paper published online on Thursday, February 3, 2011, Yale University School of Medicine researchers described in detail how Salmonella is able to make these proteins line up in just the right sequence to invade host cells. "These mechanisms present us with novel targets that might form the basis for the development of an entirely new class of anti-microbials," said Professor Dr. Jorge Galan, senior author of the paper and the Lucille P. Markey Professor of Microbial Pathogenesis and chair of the Section of Microbial Pathogenesis at Yale. In the new National Institutes of Health (NIH)-funded study, Galan and colleagues (Maria Lara-Tejero, Junya Kato, Samuel Wagner, and Xiaoyun Liu) identify what they call a bacterial sorting platform, which attracts needed proteins and lines them up in a specific order. If the proteins do not line up properly, *Salmonella*, as well as many other bacterial pathogens, cannot "inject" them into host cells to commandeer host cell functions, the lab has found. Understanding how this machine works raises the possibility that new therapies can be developed which disable this protein delivery machine and therefore thwart the ability of the bacterium to become pathogenic. The process would not kill the bacteria as most antibiotics do, but would cripple its ability to do harm. In theory, this means that bacteria such as *Salmonella* might not develop resistance to new therapies as quickly as they usually do to conventional antibiotics.

History

The genus *Salmonella* was named after Daniel Elmer Salmon, an American veterinary pathologist. While Theobald Smith was the actual discoverer of the type bacterium (*Salmonella enterica* var. *choleraesuis*) in 1885, Dr. Salmon was the administrator of the USDA research program, and thus the organism was named after him. Smith and Salmon had been searching for the cause of common hog cholera and proposed this organism as the causal agent. Later research, however, would show that this organism (now known as *Salmonella enterica*) rarely causes enteric symptoms in pigs, and was thus not the agent they were seeking (which was eventually shown to be a virus). However, related bacteria in the genus *Salmonella* were eventually shown to cause other important infectious diseases.

Salmonella nomenclature

Salmonella nomenclature is complicated. Initially, each *Salmonella* species was named according to clinical considerations, e.g., *Salmonella typhi-murium* (mouse typhoid fever), *S. cholerae-suis* (hog cholera). After it was recognized that host specificity did not exist for many species, new strains (or serovar, short for serological variants) received species names according to the location at which the new strain was isolated. Later, molecular findings led to the hypothesis that *Salmonella* consisted of only one species, *S. enterica*, and the serovar were classified into six groups, two of which are medically relevant. But as this now formalized nomenclature is not in harmony with the traditional usage familiar to specialists in microbiology and infectologists, the traditional nomenclature is common. Currently, there are two recognized species: *S. enterica* and *S. bongori*, with six main subspecies: *enterica* (I), *salamae* (II), *arizonae* (IIIa), *diarizonae* (IIIb), *houtenae* (IV), and *indica* (VI). Historically, serotype (V) was *bongori*, which is now considered its own species. The serovar classification of *Salmonella* is based on the Kauffman-White classification scheme that permits serological varieties to be differentiated from each other. Newer methods for *Salmonella* typing and subtyping include genome-based methods such as pulsed field gel electrophoresis (PFGE), Multiple Loci VNTR Analysis (MLVA), Multilocus sequence typing (MLST) and (multiplex-) PCR-based methods.

Genetics

Serovar Typhimurium has considerable diversity and may be very old. The majority of the isolates belong to a single clonal complex. Isolates are divided into phage types, but some phage types do not have a single origin as determined using mutational changes. Phage type DT104 is heterogeneous and represented in multiple sequence types, with its multidrug-resistant variant being the most successful and causing epidemics in many parts of the world.

Serovar Typhi is relatively young compared to Typhimurium, and probably originated approximately 30,000-50,000 years ago.

Sources of infection

- Unclean food, particularly in institutional kitchens and restaurants,
- Excretions from either sick or infected but apparently clinically healthy people and animals (especially endangered are caregivers and animals),
- Polluted surface water and standing water (such as in shower hoses or unused water dispensers),
- Unhygienically thawed fowl (the meltwater contains many bacteria),
- An association with reptiles (pet tortoises snakes, and frogs)(primarily aquatic turtles) is well described.

Salmonella bacteria can survive several weeks in a dry environment and several months in water; thus, they are frequently found in polluted water, contamination from the excrement of carrier animals being particularly important. Aquatic vertebrates, notably birds and reptiles, are important vectors of *Salmonella*. Poultry, cattle, and sheep frequently being agents of contamination, salmonella can be found in food, particularly meats and eggs.

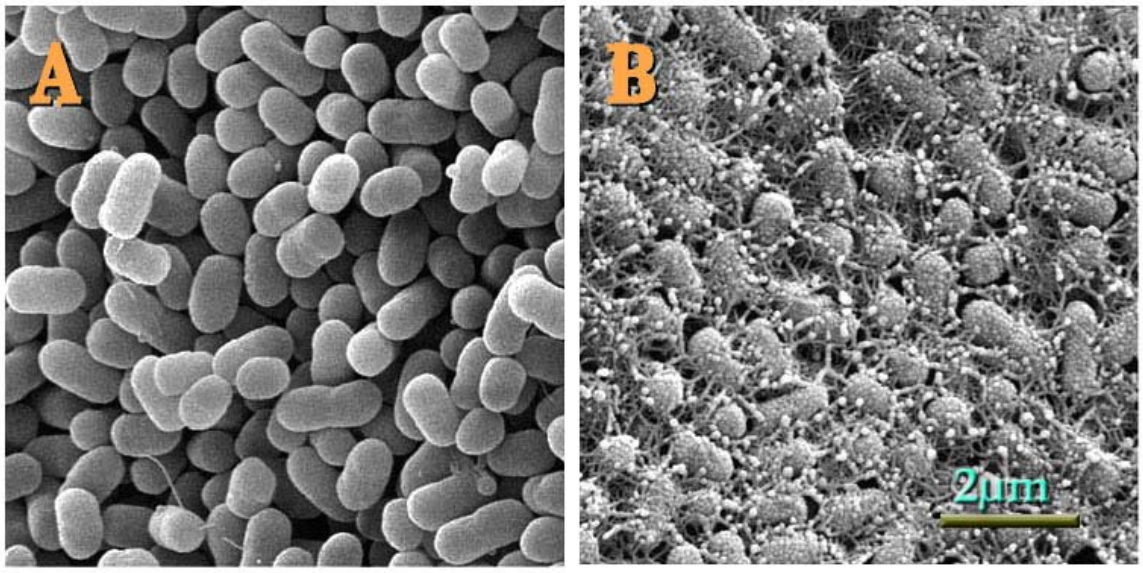
Deaths

About 142,000 (reported) Americans are infected each year with *Salmonella* Enteritidis from chicken eggs, and about 30 die. The shell of the egg may be contaminated with *Salmonella* by feces or environment, or its interior (yolk) may be contaminated by penetration of the bacteria through the porous shell or from a hen whose infected ovaries contaminate the egg during egg formation. Nevertheless, such interior egg yolk contamination is theoretically unlikely. Even under natural conditions, the rate of infection was found to be very small (0.6% in a study of naturally-contaminated eggs and 3.0% among artificially- and heavily-infected hens).

Chapter 11

Escherichia Coli O157:H7

E. Coli 0157:H7



Topographical images of colonies of *E. coli* O157:H7 strains (A) 43895OW (*curli* non-producing) and (B) 43895OR (*curli* producing) grown on agar for 48 h at 28ºC.

Escherichia coli O157:H7

ICD-10 A04.3

ICD-9 008.04

***Escherichia coli* O157:H7** is an enterohemorrhagic strain of the bacterium *Escherichia coli* and a cause of foodborne illness. Infection often leads to hemorrhagic diarrhea, and

occasionally to kidney failure, especially in young children and elderly. Transmission is via the fecal-oral route, and most illness has been associated with eating undercooked, contaminated ground beef, swimming in or drinking contaminated water, and eating contaminated vegetables.

Bacteriology

E. coli serotype O157:H7 is a Gram-negative rod-shaped bacterium. The "O" in the name refers the cell wall (somatic) antigen number, whereas the "H" refers the flagella antigen. Other serotypes may cause (usually less severe) illness, but only those with the specific O157:H7 combination are reviewed here. Other bacteria may be classified by "K" or capsular antigens. (The "O" stands for *ohne Hauch* [Ger. "without huff" or "without film"]; "H" for *Hauch*; and "K" for *Kapsel*.) This is one of hundreds of serotypes of the bacterium *Escherichia coli*. While most strains are harmless and normally found in the intestines of mammals, this strain may produce Shiga-like toxins, cause severe illness, and is a member of a class of pathogenic *E. coli* known as enterohemorrhagic *Escherichia coli* or EHEC. Sometimes also referred to by their toxin producing capabilities, Verocytotoxin producing *E. coli* (VTEC) or Shiga-like Toxin producing *E. coli* (STEC).

E. coli O157:H7 was first recognized as a pathogen as a result of an outbreak of unusual gastrointestinal illness in 1982. The outbreak was traced to contaminated hamburgers, and the illness was similar to other incidents in the United States and Japan. The etiologic agent of the illness was identified as a rare O157:H7 serotype of *Escherichia coli* in 1983. This serotype had only been isolated once before, from a sick patient in 1975.

E. coli O157:H7 is markedly different from other pathogenic *E. coli*, as well. In particular, the O157:H7 serotype is negative for invasiveness (sereny test), adheres through the *E. coli* common pilus (ECP), doesn't produce heat stable or heat labile toxins and is non-hemolytic. In addition, *E. coli* O157:H7 is usually sorbitol negative whereas 93% of all E. coli ferment sorbitol. *E. coli* O157:H7 also lacks the ability to hydrolyze 4-methylumbelliferyl-β-D-glucuronide (MUG) and does not grow at 45 °C in the presence of 0.15% bile salts. Because of the latter characteristic this serotype cannot be isolated by using standard fecal coliform methods that include incubation at 45 °C.

E. coli O157:H7 serotypes are closely related, descended from a common ancestor, divergent in plasmid content more than chromosomal content, and are no more related to other shiga toxin producing strains than any other randomly chosen E. coli serotype. *E. coli* O55:H7 and *E. coli* O157:H7 are most closely related and diverged from a common pathogenic ancestor that possessed the ability to form attaching and effacing lesions. *E. coli* O157:H7 serotypes apparently arose as a result of horizontal gene transfer of virulence factors.

Among these virulence factors are a periplasmic catalase and shiga-like toxins. Shiga-like toxins are iron regulated toxins that catalytically inactivate 60S ribosomal subunits of eukaryotic cells blocking mRNA translation and causing cell death. Shiga-like toxins are

functionally identical to toxins produced by virulent *Shigella* species. Strains of *E. coli* that express shiga-like toxins gained this ability due to infection with a prophage containing the structural coding for the toxin, and non-producing strains may become infected and produce shiga-like toxins after incubation with shiga toxin positive strains. The prophage responsible seems to have infected the strain's ancestors fairly recently, as viral particles have been observed to replicate in the host if it is stressed in some way (e.g. antibiotics). The periplasmic catalase is encoded on the pO157 plasmid and is believed to be involved in virulence by providing additional oxidative protection when infecting the host.

Natural Habitat

E. coli O157:H7 can naturally be found in cow intestines. Therefore, any object that encounters cow feces could potentially be contaminated with *E. coli* O157:H7.

Transmission

Infection with *E. coli* O157:H7 follows ingestion of contaminated food or water, or oral contact with contaminated surfaces. It is highly virulent, with a very low infectious dose: an inoculation of fewer than 10 CFU of *E. coli* O157:H7 is sufficient to cause infection, compared to over one-million CFU for other pathogenic *E. coli* strains.

A main source of infection is undercooked ground beef; other sources include consumption of unpasteurized milk and juice, raw sprouts, lettuce, and salami, and contact with infected live animals. Waterborne transmission occurs through swimming in contaminated lakes, pools, or drinking inadequately treated water. The organism is easily transmitted from person to person and has been difficult to control in child day-care centers.

E. coli O157:H7 is found on cattle farms and can live in the intestines of healthy cattle. The toxin requires highly specific receptors on the cells' surface in order to attach and enter the cell; species such as cattle, swine, and deer which do not carry these receptors may harbor toxigenic bacteria without any ill effect, shedding them in their feces, from which they may be spread to humans. Flesh can become contaminated during slaughter and butchering, and organisms can be thoroughly mixed into beef when it is ground into hamburger. Bacteria present on the cow's udders or on equipment may get into raw milk. Contaminated foods look, smell, and taste normal.

Another potential vector of *E. coli* O157:H7 is filth flies (which includes house flies, *Musca domestica*). Filth flies have been shown to be vectors of *E. coli* O157:H7 using PCR.

There have been several unsuccessful efforts to control the spread of this illness by food advocates by promotion of the so-called "Kevin's Law". This law would give the FDA

power to shutdown food processing plants that fail multiple inspections. This law has been vigorously opposed by the food processing industry.

Signs and symptoms

E. coli O157:H7 infection often causes severe, acute hemorrhagic diarrhea (although non-hemorrhagic diarrhea is also possible) and abdominal cramps. Usually little or no fever is present, and the illness resolves in 5 to 10 days. It can also be asymptomatic.

In some people, particularly children under 5 years of age and the elderly, the infection can cause haemolytic uremic syndrome (HUS), in which the red blood cells are destroyed and the kidneys fail. About 2–7% of infections lead to this complication. In the United States, haemolytic uremic syndrome is the principal cause of acute kidney failure in children, and most cases of HUS are caused by *E. coli* O157:H7.

Diagnosis

A stool culture can detect the bacterium, although it is not a routine test and so must be specifically requested. The sample is cultured on sorbitol-MacConkey (SMAC) agar, or the variant cefeximine potassium tellurite sorbitol-MacConkey agar (CT-SMAC). On SMAC agar O157 colonies appear clear due to their inability (unlike other E. coli serotypes) to ferment sorbitol. Non-sorbitol fermenting colonies are tested for the somatic O157 antigen before being confirmed as E. coli O157. Like all cultures, diagnosis is slow using this method, and swifter diagnosis is possible using PCR techniques. Newer technologies using fluorescent and antibody detection are also under development.

Surveillance

E. coli O157:H7 infection is nationally reportable in the USA and Great Britain, and is reportable in most states.

Treatment

Most people recover without antibiotics or other specific treatment in 5–10 days. There is no evidence that antibiotics improve the course of disease, and it is thought that treatment with some antibiotics may precipitate kidney complications. Antidiarrheal agents, such as loperamide (imodium), should also be avoided.

Hemolytic-uremic syndrome is a life-threatening condition usually treated in an intensive care unit. Blood transfusions and kidney dialysis are often required. With intensive care, the death rate for hemolytic uremic syndrome is 3–5%.

Costs

The pathogen results in an estimated 2,100 hospitalizations annually in the United States. The illness is often misdiagnosed; therefore, expensive and invasive diagnostic procedures may be performed. Patients who develop HUS often require prolonged hospitalization, dialysis, and long-term followup.

Prevention

Agricultural

Beef processing is a common point of contamination: during the slaughtering process, the contents of intestines or fecal material on the hide could mix with the meat, thus allowing bacteria to flourish in the warm, damp conditions. If the infected parts are then ground, the bacteria go from the surface of the cut to the interior of the ground mass. Ground beef is therefore much more likely to be a source of infection than steak. In steak only the surface area of a cut is exposed during butchering, and cooking the outside affects the entire exposed portion. In ground beef, however, bacteria are mixed throughout the meat mass, requiring the entire mass to be heated thoroughly to eliminate the pathogen. Additionally, in the production of ground beef, meat from multiple cattle is often ground together, enabling contamination from a single animal to contaminate an entire lot of ground beef.

Accordingly, reduction of infection requires preventive measures that either reduce the number of cattle that carry *E. coli* O157:H7 or reduce the contamination of meat during slaughter and grinding. These measures include careful removal and cleaning of the intestines, steam/vacuum treatment, and organic acid sprays.

In January 2007 Canadian bio-pharmaceutical company Bioniche announced it had developed a bovine vaccine capable of reducing O157:H7 in cattle by over 99%.

On March 4, 2009, the United States Department of Agriculture (USDA) granted Epitopix a conditional license for the first E. coli O157 vaccine for cattle in the US.

In May 2010, a paper from the University of Texas Southwestern Medical Center detailed preliminary results of research into the sensing mechanisms used by e. coli O157:H7 to determine when to adopt an acid-resistant state needed to escape from the digestive tract. The study introduced two different strains of e. coli lacking normal sensing mechanisms into the rumen of grain fed cattle. The strains were unable to pass through the stomach. Next, the researchers plan to conduct tests on grass fed cattle to establish if the mechanism is the same.

Culinary and dietary

Cooking all ground beef and hamburgers thoroughly, and checking the temperature using an instant-read meat thermometer, will eliminate the organism. Ground beef should be cooked until a thermometer inserted into several parts of the patty, including the thickest part, reads at least 72 °C (162 °F).

When preparing meat, it should be kept separate from other food items and all surfaces, and utensils which come into contact with raw meat should be washed thoroughly before being used again. Hand washing is similarly important. Placing cooked hamburgers or ground beef on an unwashed plate that held raw patties can transmit infection. This was the main cause of the E. coli poisoning in Scotland in 1996 that killed seven and left hundreds infected.

Unpasteurized milk, juice, and cider are potential sources of *E. coli*. Commercial juice is almost always pasteurized, and juice concentrates are also heated sufficiently to kill pathogens.

Fruits and vegetables should be washed thoroughly, especially those that will not be cooked. Children under 5 years of age, immune-compromised persons, and the elderly should avoid eating alfalfa sprouts until their safety can be assured. Methods to decontaminate alfalfa seeds and sprouts are being investigated.

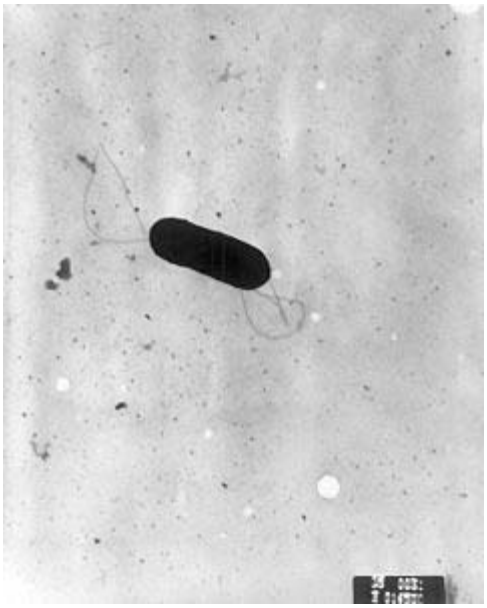
Contaminated water should be boiled at a rolling boil for at least one minute (longer at higher altitudes) before consumption. Care while swimming to avoid ingestion of potentially contaminated water can reduce the chances of infection.

Proper hand washing after using the lavatory or changing a diaper, especially among children or those with diarrhea, reduces the risk of transmission. Anyone with a diarrheal illness should avoid swimming in public pools or lakes, sharing baths with others, and preparing food for others.

Chapter 12

Listeria Monocytogenes

Listeria monocytogenes



Scanning electron micrograph of *Listeria monocytogenes*.

Scientific classification	
Kingdom:	Bacteria
Division:	Firmicutes
Class:	Bacilli
Order:	Bacillales
Family:	Listeriaceae
Genus:	*Listeria*
Species:	***L. monocytogenes***

Binomial name

Listeria monocytogenes
(Murray et al. 1926) Pirie 1940

Listeria monocytogenes is a facultative anaerobe, intracellular bacterium that is the causative agent of Listeriosis. It is one of the most virulent foodborne pathogens with 20

to 30 percent of clinical infections resulting in death. Responsible for approximately 2,500 illnesses and 500 deaths in the United States (U.S.) annually, Listeriosis is the leading cause of death among foodborne bacterial pathogens with fatality rates exceeding even Salmonella and Clostridium botulinum.

L. monocytogenes is a Gram-positive bacterium, in the division Firmicutes, named for Joseph Lister. Motile via flagella at 30 °C and below but usually not at 37 °C, *L. monocytogenes* can instead move within eukaryotic cells by explosive polymerization of actin filaments (known as *comet tails* or *actin rockets*).

Studies suggest that up to 10% of human gastrointestinal tracts may be colonized by *L. monocytogenes*.

Nevertheless, clinical diseases due to *L. monocytogenes* are more frequently recognized by veterinarians, especially as meningo-encephalitis in ruminants. See: listeriosis in animals.

Due to its frequent pathogenicity causing meningitis in newborns (acquired transvaginally), pregnant mothers are often advised not to eat soft cheeses such as Brie, Camembert, feta and queso blanco fresco, which may be contaminated with and permit growth of *L. monocytogenes*. It is the third most common cause of meningitis in newborns.

More recently, *L. monocytogenes* has been used as the model organism to illustrate the Patho-biotechnology concept.

Classification

L. monocytogenes is a gram-positive, non-spore forming, motile, facultatively anaerobic, rod-shaped bacterium. It is catalase-positive, oxidase-negative, and expresses a Beta hemolysin which causes destruction of red blood cells. This bacterium exhibits characteristic tumbling motility when viewed with light microscopy. Although *L. monocytogenes* is actively motile by means of peritrichous flagella at room temperature (20−25 °C), the organism does not synthesize flagella at body temperatures (37 °C).

The genus *Listeria* belongs to the *Clostridium* sub-branch, together with *Staphylococcus*, *Streptococcus*, *Lactobacillus* and *Brochothrix*. The genus *Listeria* includes 6 different species (*L. monocytogenes*, *L. ivanovii*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, and *L. grayi*). Both *L. ivanovii* and *L. monocytogenes* are pathogenic in mice, but only *L. monocytogenes* is consistently associated with human illness. There are 13 serotypes of *L. monocytogenes* which can cause disease, but more than 90 percent of human isolates belong to only three serotypes: 1/2a, 1/2b, and 4b. *L. monocytogenes* serotype 4b strains are responsible for 33 to 50 percent of sporadic human cases worldwide and for all major foodborne outbreaks in Europe and North America since the 1980s.

History

L. monocytogenes was first described by E.G.D.Murray in 1926 based on six cases of sudden death in young rabbits. Murray referred to the organism as *Bacterium monocytogenes* before J.H. Harvey Pirie changed the genus name to Listeria in 1940. Although clinical descriptions of *L. monocytogenes* infection in both animals and humans were published in the 1920s, not until 1952 in East Germany was it recognized as a significant cause of neonatal sepsis and meningitis. Listeriosis in adults would later be associated with patients living with compromised immune systems, such as individuals taking immunosuppressant drugs and corticosteroids for malignancies or organ transplants, and those with HIV infection.

It wasn't until 1981, however, that *L. monocytogenes* was identified as a cause of foodborne illness. An outbreak of listeriosis in Halifax, Nova Scotia involving 41 cases and 18 deaths, mostly in pregnant women and neonates, was epidemiologically linked to the consumption of coleslaw containing cabbage that had been treated with *L. monocytogenes* contaminated raw sheep manure. Since then a number of cases of foodborne listeriosis have been reported, and *L. monocytogenes* is now widely recognized as an important hazard in the food industry.

Pathogenesis

Infection by *L. monocytogenes* causes the disease listeriosis. The manifestations of listeriosis include septicemia, meningitis (or meningoencephalitis), encephalitis, corneal ulcer, pneumonia, and intrauterine or cervical infections in pregnant women, which may result in spontaneous abortion (2nd/3rd trimester) or stillbirth. Surviving neonates of Fetomaternal Listeriosis may suffer granulomatosis 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. Gastrointestinal symptoms such as nausea, vomiting, and diarrhea may precede more serious forms of listeriosis or may be the only symptoms expressed. Gastrointestinal symptoms were epidemiologically associated with use of antacids or cimetidine. The onset time to serious forms of listeriosis is unknown but may range from a few days to three weeks. The onset time to gastrointestinal symptoms is unknown but probably exceeds 12 hours. An early study suggested that *L. monocytogenes* was unique among Gram-positive bacteria in that it possessed lipopolysaccharide, which served as an endotoxin. Later it was found to not be a true endotoxin, Listeria cell walls consistently contain lipoteichoic acids, in which a glycolipid moiety, such as a galactosyl-glucosyl-diglyceride, is covalently linked to the terminal phosphomonoester of the teichoic acid. This lipid region anchors the polymer chain to the cytoplasmic membrane. These lipoteichoic acids resemble the lipopolysaccharides of gram-negative bacteria in both structure and function, being the only amphipathic polymers at the cell surface.

The infective dose of *L. monocytogenes* varies with the strain and with the susceptibility of the victim. From cases contracted through raw or supposedly pasteurized milk, one

may safely assume that in susceptible persons, fewer than 1,000 total organisms may cause disease. *L. monocytogenes* may invade the gastrointestinal epithelium. Once the bacterium enters the host's monocytes, macrophages, or polymorphonuclear leukocytes, it becomes blood-borne (septicemic) and can grow. Its presence intracellularly in phagocytic cells also permits access to the brain and probably transplacental migration to the fetus in pregnant women. The pathogenesis of *L. monocytogenes* centers on its ability to survive and multiply in phagocytic host cells.

Regulation of pathogenesis

L. monocytogenes can act as a saprophyte or a pathogen depending on its environment. When this bacteria is present within a host organism quorum sensing causes the up regulation of several virulence genes. Depending on the location of the bacteria within the host organism different activators up regulate the virulence genes. SigB, an alternative sigma factor, up regulates Vir genes in the intestines whereas PrfA up regulates gene expression when the bacteria is present in blood. Little is known about the mechanism in how this bacteria switches between acting as a saprophyte and a pathogen however, it is thought that several non-coding RNAs are required to induce this change.

Pathogenicity of Lineages

L. monocytogenes has three distinct lineages with differing evolutionary histories and pathogenic potentials. Lineage I strains contain the majority of human clinical isolates and all human epidemic clones, but are underrepresented in animal clinical isolates. Lineage II strains are overrepresented in animal cases and underrepresented in human clinical cases as well as more prevalent in environmental and food samples. Lineage III isolates are very rare but significantly more common in animal isolates than human.

Treatment

When listeric meningitis occurs, the overall mortality may reach 70%; from septicemia 50%, from perinatal/neonatal infections greater than 80%. In infections during pregnancy, the mother usually survives. Reports of successful treatment with parenteral penicillin or ampicillin exist. Trimethoprim-sulfamethoxazole has been shown effective in patients allergic to penicillin.

Bacteriophage treatments have been developed by several companies. EBI Food Safety and Intralytix both have products suitable for treatment of the bacteria. The Food and Drug Administration of the United States approved a cocktail of six bacteriophages from Intralytix, and a one type phage product from EBI Food Safety designed to kill the bacteria *L. monocytogenes*. Uses would potentially include spraying it on fruits and ready-to-eat meat such as sliced ham and turkey.

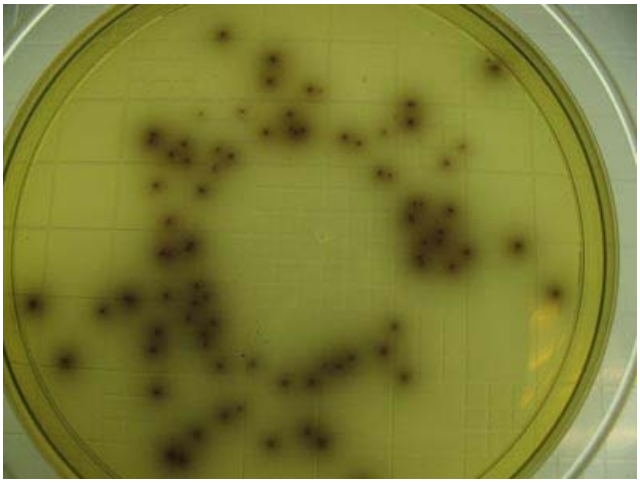
Use as a transfection vector

Because *L. monocytogenes* is an intracellular parasite, some studies have used this bacterium as a vector to deliver genes *in vitro.* Current transfection efficiency remains poor. One example of the successful use of *L. monocytogenes* in *in vitro* transfer technologies is in the delivery of gene therapies for cystic fibrosis cases.

Cancer vaccine

A live attenuated *L. monocytogenes* cancer vaccine named ADXS11-001 is under development as a possible treatment for cervical carcinoma.

Detection



Colonies of typical *Listeria monocytogenes* as they appear when grown on Listeria selective agar

The methods for analysis of food are complex and time-consuming. The present U.S. Food and Drug Administration (FDA) method, revised in September, 1990, requires 24 and 48 hours of enrichment, followed by a variety of other tests. Total time to identification takes from 5 to 7 days, but the announcement of specific nonradiolabled DNA probes should soon allow a simpler and faster confirmation of suspect isolates.

Recombinant DNA technology may even permit 2-to-3 day positive analysis in the future. Currently, the FDA is collaborating in adapting its methodology to quantitate very low numbers of the organisms in foods.

Epidemiology

Researchers have found *L. monocytogenes* in at least 37 mammalian species, both domesticated and feral, as well as in at least 17 species of birds and possibly in some

species of fish and shellfish. Laboratories can isolate *L. monocytogenes* from soil, silage, and other environmental sources. *L. monocytogenes* is quite hardy and resists the deleterious effects of freezing, drying, and heat remarkably well for a bacterium that does not form spores. Most *L. monocytogenes* are pathogenic to some degree.

Routes of infection

L. monocytogenes has been associated with such foods as raw milk, pasteurized fluid milk, cheeses (particularly soft-ripened varieties), ice cream, raw vegetables, fermented raw-meat sausages, raw and cooked poultry, raw meats (of all types), and raw and smoked fish. Its ability to grow at temperatures as low as 0°C permits multiplication in refrigerated foods. In refrigeration temperature such as 4°C the amount of ferric iron promotes the growth of *L. monocytogenes*.

Infectious cycle

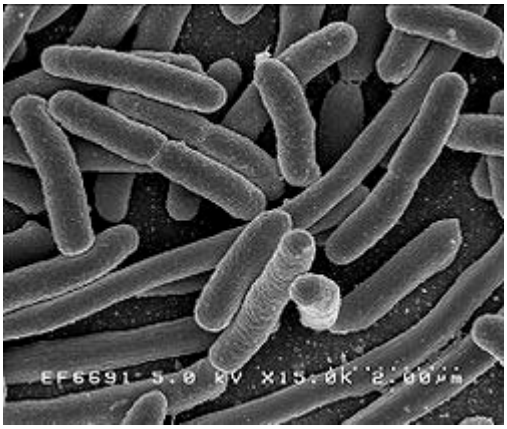
The primary site of infection is the intestinal epithelium where the bacteria invade non-phagocytic cells via the "zipper" mechanism. Uptake is stimulated by the binding of listerial internalins (Inl) to host cell adhesion factors such as E-cadherin or Met. This binding activates certain Rho-GTPases which subsequently bind and stabilize Wiskott Aldrich Syndrome Protein (WASp). WASp can then bind the Arp2/3 complex and serve as an actin nucleation point. Subsequent actin polymerization extends the cell membrane around the bacterium, eventually engulfing it. The net effect of internalin binding is to exploit the junction forming-apparatus of the host into internalizing the bacterium. Note that *L. monocytogenes* can also invade phagocytic cells (e.g. macrophages) but only requires internalins for invasion of non-phagocytic cells.

Following internalisation, the bacterium must escape from the vacuole/phagosome before fusion with a lysosome can occur. Three main virulence factors which allow the bacterium to escape are listeriolysin O (LLO - encoded by *hly*) phospholipase A (encoded by *plcA*) and phospholipase B (*plcB*). Secretion of LLO and PlcB disrupts the vacuolar membrane and allows the bacterium to escape into the cytoplasm where it may proliferate.

Once in the cytoplasm, *L. monocytogenes* exploits host actin for the second time. ActA proteins associated with the old bacterial cell pole (being a bacillus, *L. monocytogenes* septates in the middle of the cell and thus has one new pole and one old pole) are capable of binding the Arp2/3 complex and thus induce actin nucleation at a specific area of the bacterial cell surface. Actin polymerization then propels the bacterium unidirectionally into the host cell membrane. The protrusion which is formed may then be internalised by a neighbouring cell, forming a double-membrane vacuole from which the bacterium must escape using LLO and PlcB.

Chapter 13

Escherichia Coli

Escherichia coli

Scientific classification
Domain: Bacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Enterobacteriales
Family: Enterobacteriaceae
Genus: *Escherichia*
Species: ***E. coli***
Binomial name
Escherichia coli (Migula 1895) Castellani and Chalmers 1919
Synonyms
Bacillus coli communis Escherich 1885

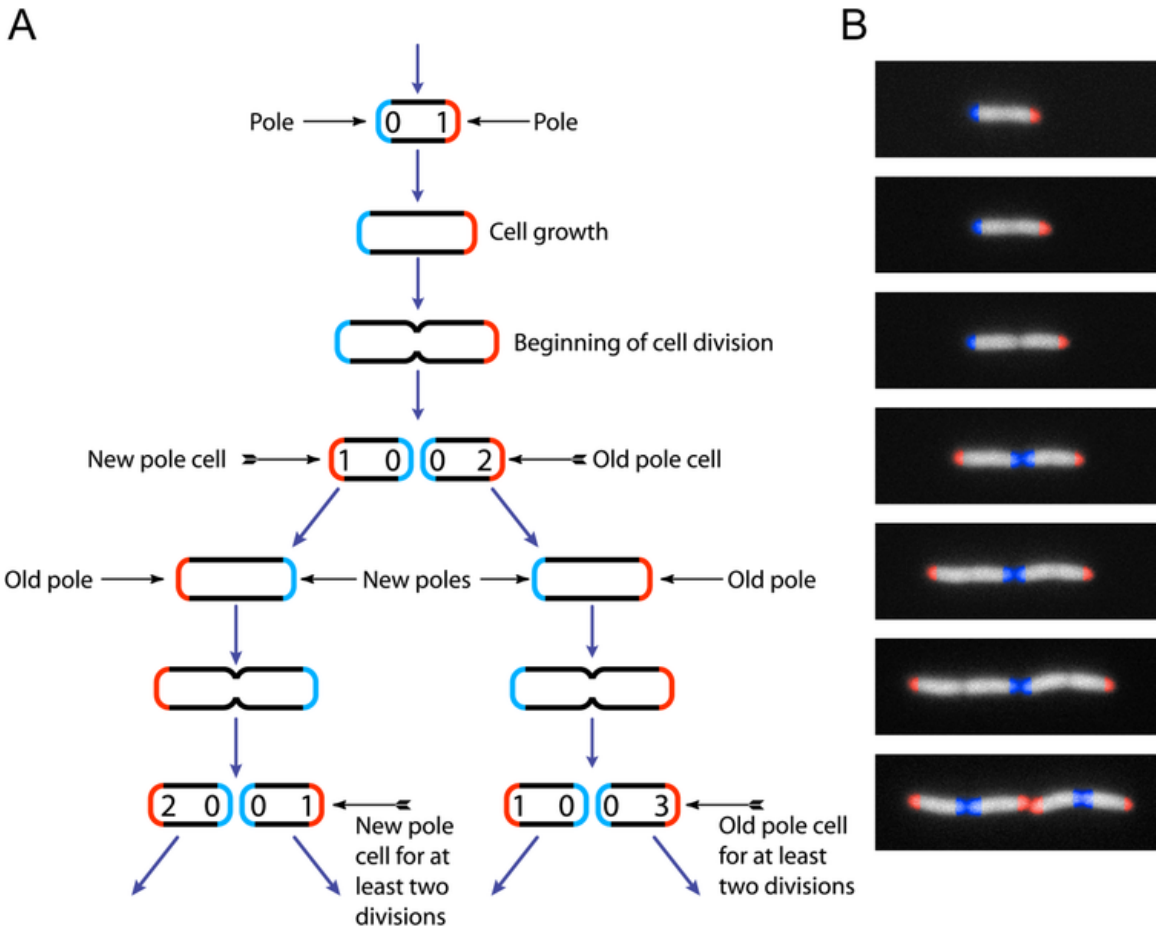
Escherichia coli is a Gram-negative rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Most *E. coli* strains are harmless, but some, such as serotype O157:H7, can cause serious food poisoning in humans, and are occasionally responsible for product recalls. The harmless strains are

part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2, and by preventing the establishment of pathogenic bacteria within the intestine.

E. coli are not always confined to the intestine, and their ability to survive for brief periods outside the body makes them an ideal indicator organism to test environmental samples for fecal contamination. The bacteria can also be grown easily and its genetics are comparatively simple and easily manipulated or duplicated through a process of metagenics, making it one of the best-studied prokaryotic model organisms, and an important species in biotechnology and microbiology.

E. coli was discovered by German paediatrician and bacteriologist Theodor Escherich in 1885, and is now classified as part of the Enterobacteriaceae family of gamma-proteobacteria.

Biology and biochemistry



Model of successive binary fission in *E. coli*

E. coli is Gram-negative, facultative anaerobic and non-sporulating. Cells are typically rod-shaped and are about 2 micrometres (µm) long and 0.5 µm in diameter, with a cell

volume of 0.6 - 0.7 $(\mu m)^3$. It can live on a wide variety of substrates. *E. coli* uses mixed-acid fermentation in anaerobic conditions, producing lactate, succinate, ethanol, acetate and carbon dioxide. Since many pathways in mixed-acid fermentation produce hydrogen gas, these pathways require the levels of hydrogen to be low, as is the case when *E. coli* lives together with hydrogen-consuming organisms such as methanogens or sulphate-reducing bacteria.

Optimal growth of *E. coli* occurs at 37°C (98.6°F) but some laboratory strains can multiply at temperatures of up to 49°C (120.2°F). Growth can be driven by aerobic or anaerobic respiration, using a large variety of redox pairs, including the oxidation of pyruvic acid, formic acid, hydrogen and amino acids, and the reduction of substrates such as oxygen, nitrate, dimethyl sulfoxide and trimethylamine N-oxide.

Strains that possess flagella can swim and are motile. The flagella have a peritrichous arrangement.

E. coli and related bacteria possess the ability to transfer DNA via bacterial conjugation, transduction or transformation, which allows genetic material to spread horizontally through an existing population. This process led to the spread of the gene encoding shiga toxin from *Shigella* to E. coli O157:H7, carried by a bacteriophage.

Diversity

As more is known about certain organisms, such as genetic information, the taxonomic classification of species is changed to reflect the advance in knowledge, however in the case of Escherichia coli due to its medical importance, this has not occurred (namely split into several genera/species) and remains one of the most diverse bacterial species: only 20% of the genome is common to all strains. In fact, from the evolutionary point of view, the members of genus Shigella (*dysenteriae*, *flexneri*, *boydii*, *sonnei*) are actually E. coli strains "in disguise" (i.e. E.coli is paraphyletic to the genus).

A strain of *E. coli* is a sub-group within the species that has unique characteristics that distinguish it from other *E. coli* strains. These differences are often detectable only at the molecular level; however, they may result in changes to the physiology or lifecycle of the bacterium. For example, a strain may gain pathogenic capacity, the ability to use a unique carbon source, the ability to take upon a particular ecological niche or the ability to resist antimicrobial agents. Different strains of *E. coli* are often host-specific, making it possible to determine the source of faecal contamination in environmental samples. For example, knowing which *E. coli* strains are present in a water sample allows researchers to make assumptions about whether the contamination originated from a human, another mammal or a bird.

New strains of *E. coli* evolve through the natural biological process of mutation and through horizontal gene transfer. Some strains develop traits that can be harmful to a host animal. These virulent strains typically cause a bout of diarrhoea that is unpleasant in healthy adults and is often lethal to children in the developing world. More virulent

strains, such as O157:H7 cause serious illness or death in the elderly, the very young or the immunocompromised.

E. coli is the type species of the genus and the type strain is ATCC 11775.

A common subdivison system of E.coli, but not based on evolutionary relatedness, is by serotype, which is based on major surface antigens (O antigen: part of lipopolysaccharide layer; H: flagellin; K antigen: capsule), e.g. O157:H7) (NB: K-12, the common laboratory strain is not a serotype.)

Role as normal microbiota

E. coli normally colonizes an infant's gastrointestinal tract within 40 hours of birth, arriving with food or water or with the individuals handling the child. In the bowel, it adheres to the mucus of the large intestine. It is the primary facultative anaerobe of the human gastrointestinal tract. (Facultative anaerobes are organisms that can grow in either the presence or absence of oxygen.) As long as these bacteria do not acquire genetic elements encoding for virulence factors, they remain benign commensals.

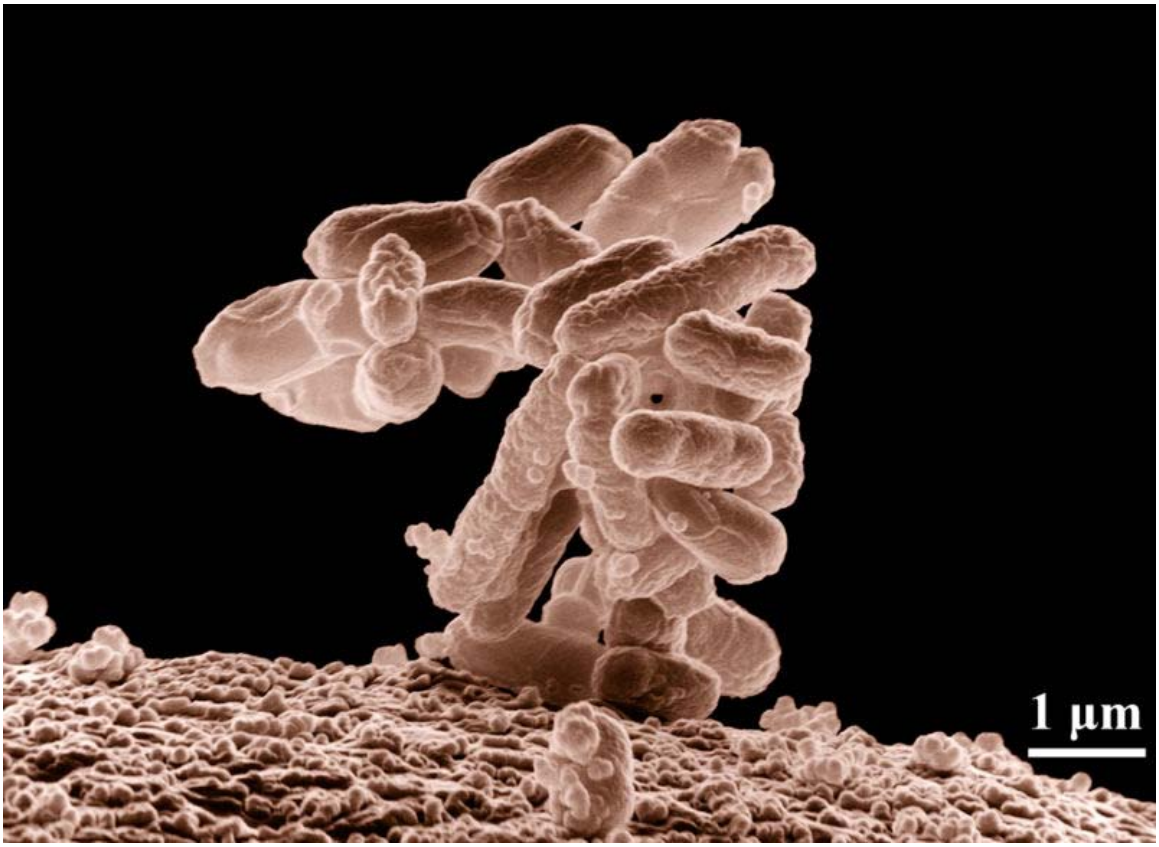
Therapeutic use of nonpathogenic *E. coli*

Nonpathogenic *Escherichia coli* strain Nissle 1917 also known as Mutaflor is used as a probiotic agent in medicine, mainly for the treatment of various gastroenterological diseases, including inflammatory bowel disease.

Role in disease

Virulent strains of *E. coli* can cause gastroenteritis, urinary tract infections, and neonatal meningitis. In rarer cases, virulent strains are also responsible for haemolytic-uremic syndrome, peritonitis, mastitis, septicaemia and Gram-negative pneumonia.

Gastrointestinal infection



Low-temperature electron micrograph of a cluster of *E. coli* bacteria, magnified 10,000 times. Each individual bacterium is a rounded cylinder.

Certain strains of *E. coli*, such as O157:H7, O121 and O104:H21, produce potentially lethal toxins. Food poisoning caused by *E. coli* is usually caused by eating unwashed vegetables or undercooked meat. O157:H7 is also notorious for causing serious and even life-threatening complications such as haemolytic-uremic syndrome. This particular strain is linked to the 2006 United States *E. coli* outbreak due to fresh spinach. Severity of the illness varies considerably; it can be fatal, particularly to young children, the elderly or the immunocompromised, but is more often mild. Earlier, poor hygienic methods of preparing meat in Scotland killed seven people in 1996 due to *E. coli* poisoning, and left hundreds more infected. *E. coli* can harbour both heat-stable and heat-labile enterotoxins. The latter, termed LT, contains one A subunit and five B subunits arranged into one holotoxin, and is highly similar in structure and function to cholera toxins. The B subunits assist in adherence and entry of the toxin into host intestinal cells, while the A subunit is cleaved and prevents cells from absorbing water, causing diarrhea. LT is secreted by the Type 2 secretion pathway.

If *E. coli* bacteria escape the intestinal tract through a perforation (for example from an ulcer, a ruptured appendix, or due to a surgical error) and enter the abdomen, they usually cause peritonitis that can be fatal without prompt treatment. However, *E. coli* are

extremely sensitive to such antibiotics as streptomycin or gentamicin. This could change since, as noted below, *E. coli* quickly acquires drug resistance. Recent research suggests that treatment with antibiotics does not improve the outcome of the disease, and may in fact significantly increase the chance of developing haemolytic-uremic syndrome.

Intestinal mucosa-associated *E. coli* are observed in increased numbers in the inflammatory bowel diseases, Crohn's disease and ulcerative colitis. Invasive strains of *E. coli* exist in high numbers in the inflamed tissue, and the number of bacteria in the inflamed regions correlates to the severity of the bowel inflammation.

Virulence properties

Enteric *E. coli* (EC) are classified on the basis of serological characteristics and virulence properties. Virotypes include:

Name	Hosts	Description
Enterotoxigenic *E. coli* (ETEC)	causative agent of diarrhea (without fever) in humans, pigs, sheep, goats, cattle, dogs, and horses	ETEC uses fimbrial adhesins (projections from the bacterial cell surface) to bind enterocyte cells in the small intestine. ETEC can produce two proteinaceous enterotoxins: • the larger of the two proteins, **LT enterotoxin**, is similar to cholera toxin in structure and function. • the smaller protein, **ST enterotoxin** causes cGMP accumulation in the target cells and a subsequent secretion of fluid and electrolytes into the intestinal lumen. ETEC strains are non-invasive, and they do not leave the intestinal lumen. ETEC is the leading bacterial cause of diarrhoea in children in the developing world, as well as the most common cause of traveler's diarrhea. Each year, ETEC causes more than 200 million cases of diarrhoea and 380,000 deaths, mostly in children in developing countries.
Enteropathogenic *E. coli* (EPEC)	causative agent of diarrhea in humans, rabbits, dogs, cats and horses	Like ETEC, EPEC also causes diarrhoea, but the molecular mechanisms of colonization and aetiology are different. EPEC lack fimbriae, ST and LT toxins, but they utilize an adhesin known as intimin to bind host intestinal cells. This virotype has an array of virulence factors that are similar to those found in *Shigella*, and

		may possess a shiga toxin. Adherence to the intestinal mucosa causes a rearrangement of actin in the host cell, causing significant deformation. EPEC cells are moderately invasive (i.e. they enter host cells) and elicit an inflammatory response. Changes in intestinal cell ultrastructure due to "attachment and effacement" is likely the prime cause of diarrhoea in those afflicted with EPEC.
Enteroinvasive *E. coli* *(EIEC)*	found only in humans	EIEC infection causes a syndrome that is identical to Shigellosis, with profuse diarrhoea and high fever.
Enterohemorrhagic *E. coli* (EHEC)	found in humans, cattle, and goats	The most famous member of this virotype is strain O157:H7, which causes bloody diarrhoea and no fever. EHEC can cause hemolytic-uremic syndrome and sudden kidney failure. It uses bacterial fimbriae for attachment (E. coli common pilus, ECP), is moderately invasive and possesses a phage-encoded Shiga toxin that can elicit an intense inflammatory response.
Enteroaggregative *E. coli* (EAEC)	found only in humans	So named because they have fimbriae which aggregate tissue culture cells, EAEC bind to the intestinal mucosa to cause watery diarrhea without fever. EAEC are non-invasive. They produce a hemolysin and an ST enterotoxin similar to that of ETEC.

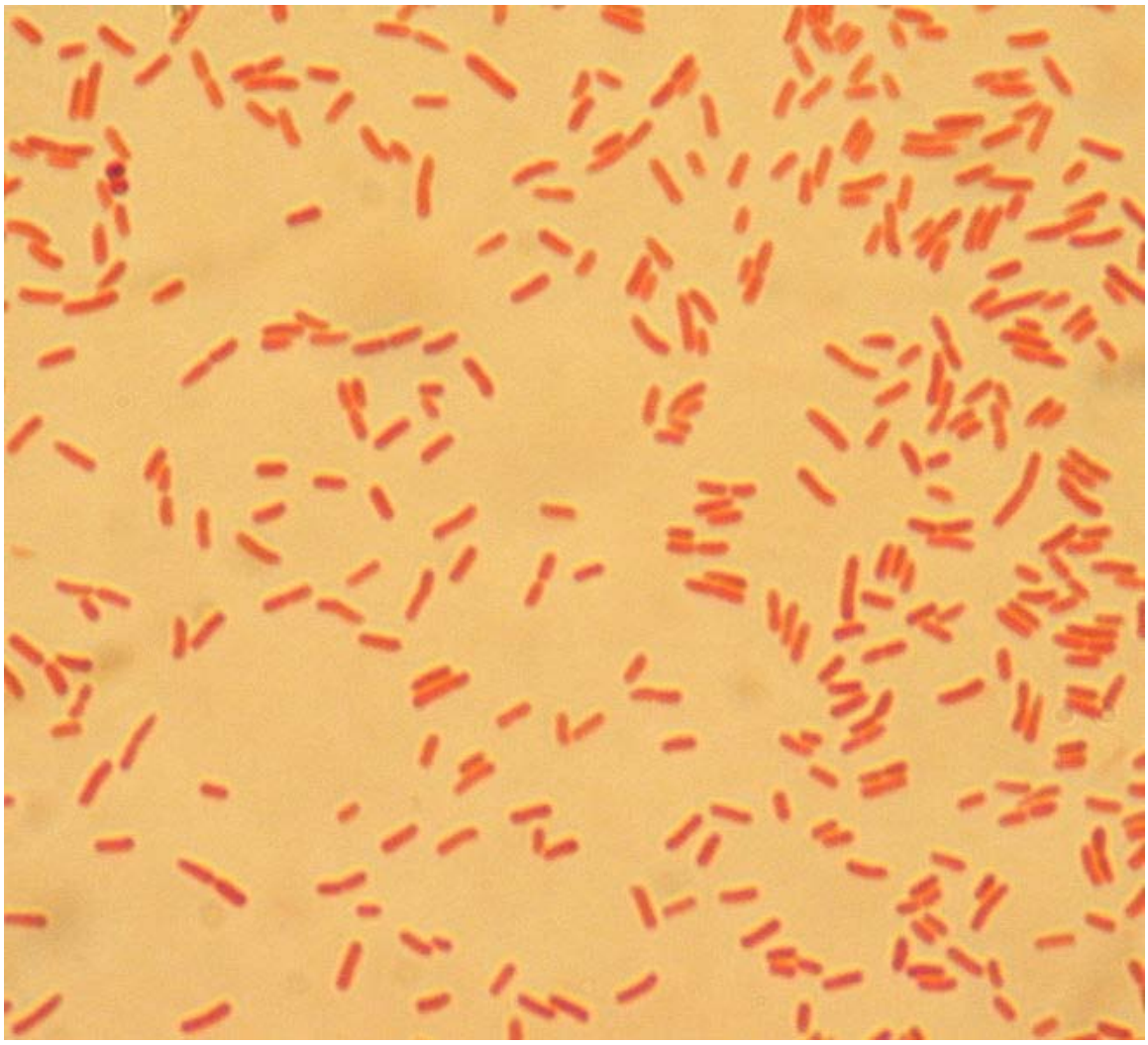
Epidemiology of gastrointestinal infection

Transmission of pathogenic *E. coli* often occurs via faecal-oral transmission. Common routes of transmission include: unhygienic food preparation, farm contamination due to manure fertilization, irrigation of crops with contaminated greywater or raw sewage, feral pigs on cropland, or direct consumption of sewage-contaminated water. Dairy and beef cattle are primary reservoirs of *E. coli* O157:H7, and they can carry it asymptomatically and shed it in their faeces. Food products associated with *E. coli* outbreaks include raw ground beef, raw seed sprouts or spinach, raw milk, unpasteurized juice, unpasteurized cheese and foods contaminated by infected food workers via faecal-oral route.

According to the U.S. Food and Drug Administration, the faecal-oral cycle of transmission can be disrupted by cooking food properly, preventing cross-contamination, instituting barriers such as gloves for food workers, instituting health care policies so food industry employees seek treatment when they are ill, pasteurization of juice or dairy products and proper hand washing requirements.

Shiga toxin-producing *E. coli* (STEC), specifically serotype O157:H7, have also been transmitted by flies, as well as direct contact with farm animals, petting zoo animals, and airborne particles found in animal-rearing environments.

Urinary tract infection



E. coli bacteria, the most prevalent gram-negative flora in the intestine

Uropathogenic *E. coli* (UPEC) is responsible for approximately 90% of urinary tract infections (UTI) seen in individuals with ordinary anatomy. In *ascending infections*, fecal bacteria colonize the urethra and spread up the urinary tract to the bladder as well as to the kidneys (causing pyelonephritis), or the prostate in males. Because women have a shorter urethra than men, they are 14-times more likely to suffer from an ascending UTI.

Uropathogenic *E. coli* utilize P fimbriae (pyelonephritis-associated pili) to bind urinary tract endothelial cells and colonize the bladder. These adhesins specifically bind D-galactose-D-galactose moieties on the P blood-group antigen of erythrocytes and

uroepithelial cells. Approximately 1% of the human population lacks this receptor, and its presence or absence dictates an individual's susceptibility to *E. coli* urinary tract infections. Uropathogenic *E. coli* produce alpha- and beta-hemolysins, which cause lysis of urinary tract cells.

UPEC can evade the body's innate immune defences (e.g. the complement system) by invading superficial umbrella cells to form intracellular bacterial communities (IBCs). They also have the ability to form K antigen, capsular polysaccharides that contribute to biofilm formation. Biofilm-producing *E. coli* are recalcitrant to immune factors and antibiotic therapy and are often responsible for chronic urinary tract infections. K antigen-producing *E. coli* infections are commonly found in the upper urinary tract.

Descending infections, though relatively rare, occur when *E. coli* cells enter the upper urinary tract organs (kidneys, bladder or ureters) from the blood stream.

Neonatal meningitis

It is produced by a serotype of *Escherichia coli* that contains a capsular antigen called K1. The colonisation of the newborn's intestines with these stems, that are present in the mother's vagina, lead to bacteraemia, which leads to meningitis. And because of the absence of the IgM antibodies from the mother (these do not cross the placenta because FcRn only mediates the transfer of IgG), plus the fact that the body recognises as self the K1 antigen, as it resembles the cerebral glicopeptides, this leads to a severe meningitis in the neonates.

Laboratory diagnosis

In stool samples microscopy will show Gram negative rods, with no particular cell arrangement. Then, either MacConkey agar or EMB agar (or both) are inoculated with the stool. On MacConkey agar, deep red colonies are produced as the organism is lactose-positive, and fermentation of this sugar will cause the medium's pH to drop, leading to darkening of the medium. Growth on Levine EMB agar produces black colonies with greenish-black metallic sheen. This is diagnostic of *E. coli*. The organism is also lysine positive, and grows on TSI slant with a (A/A/g+/H_2S-) profile. Also, IMViC is {+ + - -} for *E. coli*; as it's indole-positive (red ring) and methyl red-positive (bright red), but VP-negative (no change-colourless) and citrate-negative (no change-green colour). Tests for toxin production can use mammalian cells in tissue culture, which are rapidly killed by shiga toxin. Although sensitive and very specific, this method is slow and expensive.

Typically diagnosis has been done by culturing on sorbitol-MacConkey medium and then using typing antiserum. However, current latex assays and some typing antisera have shown cross reactions with non-*E. coli* O157 colonies. Furthermore, not all *E. coli* O157 strains associated with HUS are nonsorbitol fermentors.

The Council of State and Territorial Epidemiologists recommend that clinical laboratories screen at least all bloody stools for this pathogen. The American Gastroenterological

Association Foundation (AGAF) recommended in July 1994 that all stool specimens should be routinely tested for *E. coli* O157:H7. It is recommended that the clinician check with their state health department or the Centers for Disease Control and Prevention to determine which specimens should be tested and whether the results are reportable.

Other methods for detecting *E. coli* O157 in stool include ELISA tests, colony immunoblots, direct immunofluorescence microscopy of filters, as well as immunocapture techniques using magnetic beads. These assays are designed as screening tool to allow rapid testing for the presence of *E. coli* O157 without prior culturing of the stool specimen.

Antibiotic therapy and resistance

Bacterial infections are usually treated with antibiotics. However, the antibiotic sensitivities of different strains of *E. coli* vary widely. As Gram-negative organisms, *E. coli* are resistant to many antibiotics that are effective against Gram-positive organisms. Antibiotics which may be used to treat *E. coli* infection include amoxicillin as well as other semi-synthetic penicillins, many cephalosporins, carbapenems, aztreonam, trimethoprim-sulfamethoxazole, ciprofloxacin, nitrofurantoin and the aminoglycosides.

Antibiotic resistance is a growing problem. Some of this is due to overuse of antibiotics in humans, but some of it is probably due to the use of antibiotics as growth promoters in food of animals. A study published in the journal *Science* in August 2007 found that the rate of adaptative mutations in *E. coli* is "on the order of 10^{-5} per genome per generation, which is 1,000 times as high as previous estimates," a finding which may have significance for the study and management of bacterial antibiotic resistance.

Antibiotic-resistant *E. coli* may also pass on the genes responsible for antibiotic resistance to other species of bacteria, such as *Staphylococcus aureus*, through a process called horizontal gene transfer. *E. coli* often carry multidrug resistant plasmids and under stress readily transfer those plasmids to other species. Indeed, *E. coli* is a frequent member of biofilms where many species of bacteria exist in close proximity to each other. This mixing of species allows *E. coli* strains that are piliated to accept and transfer plasmids from and to other bacteria. Thus *E. coli* and the other enterobacteria are important reservoirs of transferable antibiotic resistance.

Beta-lactamase strains

Resistance to beta-lactam antibiotics has become a particular problem in recent decades, as strains of bacteria that produce extended-spectrum beta-lactamases have become more common. These beta-lactamase enzymes make many, if not all, of the penicillins and cephalosporins ineffective as therapy. Extended-spectrum beta-lactamase–producing *E. coli* are highly resistant to an array of antibiotics and infections by these strains are difficult to treat. In many instances, only two oral antibiotics and a very limited group of intravenous antibiotics remain effective. In 2009, a gene called New Delhi metallo-beta-

lactamase (shortened NDM-1) that even gives resistance to intravenous antibiotic carbapenem, were discovered in India and Pakistan on *E. coli* bacteria.

Increased concern about the prevalence of this form of "superbug" in the United Kingdom has led to calls for further monitoring and a UK-wide strategy to deal with infections and the deaths. Susceptibility testing should guide treatment in all infections in which the organism can be isolated for culture.

Phage therapy

Phage therapy—viruses that specifically target pathogenic bacteria—has been developed over the last 80 years, primarily in the former Soviet Union, where it was used to prevent diarrhoea caused by *E. coli*. Presently, phage therapy for humans is available only at the Phage Therapy Center in the Republic of Georgia and in Poland. However, on January 2, 2007, the United States FDA gave Omnilytics approval to apply its *E. coli* O157:H7 killing phage in a mist, spray or wash on live animals that will be slaughtered for human consumption. The Bacteriophage T4 is a highly studied phage that targets E. coli for infection.

Vaccination

Researchers have actively been working to develop safe, effective vaccines to lower the worldwide incidence of *E. coli* infection. In March 2006, a vaccine eliciting an immune response against the *E. coli* O157:H7 O-specific polysaccharide conjugated to recombinant exotoxin A of *Pseudomonas aeruginosa* (O157-rEPA) was reported to be safe in children two to five years old. Previous work had already indicated that it was safe for adults. A phase III clinical trial to verify the large-scale efficacy of the treatment is planned.

In 2006 Fort Dodge Animal Health (Wyeth) introduced an effective live attenuated vaccine to control airsacculitis and peritonitis in chickens. The vaccine is a genetically modified avirulent vaccine that has demonstrated protection against O78 and untypeable strains.

In January 2007 the Canadian bio-pharmaceutical company Bioniche announced it has developed a cattle vaccine which reduces the number of O157:H7 shed in manure by a factor of 1000, to about 1000 pathogenic bacteria per gram of manure.

In April 2009 a Michigan State University researcher announced that he has developed a working vaccine for a strain of *E. coli*. Mahdi Saeed, professor of epidemiology and infectious disease in MSU's colleges of Veterinary Medicine and Human Medicine, has applied for a patent for his discovery and has made contact with pharmaceutical companies for commercial production.

Model organism in life science research

Role in biotechnology

Because of its long history of laboratory culture and ease of manipulation, *E. coli* also plays an important role in modern biological engineering and industrial microbiology. The work of Stanley Norman Cohen and Herbert Boyer in *E. coli*, using plasmids and restriction enzymes to create recombinant DNA, became a foundation of biotechnology.

Considered a very versatile host for the production of heterologous proteins, researchers can introduce genes into the microbes using plasmids, allowing for the mass production of proteins in industrial fermentation processes. Genetic systems have also been developed which allow the production of recombinant proteins using *E. coli*. One of the first useful applications of recombinant DNA technology was the manipulation of *E. coli* to produce human insulin. Modified *E. coli* have been used in vaccine development, bioremediation, and production of immobilised enzymes. *E. coli* cannot, however, be used to produce some of the more large, complex proteins which contain multiple disulfide bonds and, in particular, unpaired thiols, or proteins that also require post-translational modification for activity.

Studies are also being performed into programming *E. coli* to potentially solve complicated mathematics problems such as the Hamiltonian path problem.

Model organism

E. coli is frequently used as a model organism in microbiology studies. Cultivated strains (e.g. *E. coli* K12) are well-adapted to the laboratory environment, and, unlike wild type strains, have lost their ability to thrive in the intestine. Many lab strains lose their ability to form biofilms. These features protect wild type strains from antibodies and other chemical attacks, but require a large expenditure of energy and material resources.

In 1946, Joshua Lederberg and Edward Tatum first described the phenomenon known as bacterial conjugation using *E. coli* as a model bacterium, and it remains the primary model to study conjugation. *E. coli* was an integral part of the first experiments to understand phage genetics, and early researchers, such as Seymour Benzer, used *E. coli* and phage T4 to understand the topography of gene structure. Prior to Benzer's research, it was not known whether the gene was a linear structure, or if it had a branching pattern.

E. coli was one of the first organisms to have its genome sequenced; the complete genome of *E. coli* K12 was published by *Science* in 1997.

The long-term evolution experiments using *E. coli*, begun by Richard Lenski in 1988, have allowed direct observation of major evolutionary shifts in the laboratory. In this experiment, one population of *E. coli* unexpectedly evolved the ability to aerobically metabolize citrate. This capacity is extremely rare in *E. coli*. As the inability to grow aerobically is normally used as a diagnostic criterion with which to differentiate *E. coli*

from other, closely related bacteria such as *Salmonella*, this innovation may mark a speciation event observed in the lab.

By combining nanotechnologies with landscape ecology complex habitat landscapes can be generated with details at the nanoscale. On such synthetic ecosystems evolutionary experiments with *E. coli* have been performed in order to study the spatial biophysics of adaptation in an island biogeography on-chip.

Environmental quality

E. coli bacteria have been commonly found in recreational waters and their presence is used to indicate the presence of recent faecal contamination, but *E. coli* presence may not be indicative of human waste. *E. coli* are harboured in all warm-blooded animals: birds and mammals alike. *E. coli* bacteria have also been found in fish and turtles. Sand and soil also harbor *E. coli* bacteria and some strains of *E. coli* have become naturalized. Some geographic areas may support unique populations of *E. coli* and conversely, some *E. coli* strains are cosmopolitan.

Chapter 14

Yersinia Pseudotuberculosis

Yersinia pseudotuberculosis	
Scientific classification	
Kingdom:	Bacteria
Phylum:	Proteobacteria
Class:	Gammaproteobacteria
Order:	Enterobacteriales
Family:	Enterobacteriaceae
Genus:	*Yersinia*
Species:	***Y. pseudotuberculosis***
Binomial name	
Yersinia pseudotuberculosis (Pfeiffer 1889) Smith & Thal 1965	

Yersinia pseudotuberculosis is a Gram-negative bacterium which primarily causes Pseudotuberculosis (Yersinia) disease in animals; humans occasionally get infected zoonotically, most often through the food-borne route.

Pathogenesis

Yersinia pseudotuberculosis

ICD-10	A04.8, A28.2
DiseasesDB	14237
eMedicine	article/226871
MeSH	D015012

In animals, *Y. pseudotuberculosis* can cause tuberculosis-like symptoms, including localized tissue necrosis and granulomas in the spleen, liver, and lymph node.

In humans, symptoms of Pseudotuberculosis (Yersinia) are similar to those of infection with *Yersinia enterocolitica* (fever and right-sided abdominal pain), except that the diarrheal component is often absent, which sometimes makes the resulting condition difficult to diagnose. *Y. pseudotuberculosis* infections can mimic appendicitis, especially in children and younger adults, and, in rare cases the disease may cause skin complaints (erythema nodosum), joint stiffness and pain (reactive arthritis), or spread of bacteria to the blood (bacteremia).

Pseudotuberculosis (Yersinia) usually becomes apparent 5–10 days after exposure and typically lasts 1–3 weeks without treatment. In complex cases or those involving immunocompromised patients, antibiotics may be necessary for resolution; ampicillin, aminoglycosides, tetracycline, chloramphenicol, or a cephalosporin may all be effective.

The recently described syndrome *Izumi-fever* has been linked to infection with *Y.pseudotuberculosis*.

The symptoms of fever and abdominal pain mimicking appendicitis (actually from mesenteric lymphadenitis) associated with *Y. pseudotuberculosis* infection are not typical of the diarrhea and vomiting from classical food poisoning incidents. Although *Y. pseudotuberculosis* is usually only able to colonize hosts by peripheral routes and cause serious disease in immunocompromised individuals, if this bacterium gains access to the blood stream, it has an LD_{50} comparable to *Y. pestis* at only 10CFU.

Virulence Factors

To facilitate attachment, invasion, and colonization of its host, this bacterium possesses many virulence factors. Superantigens, bacterial adhesions, and the actions of Yops (which are bacterial proteins once thought to be "*Yersinia* outer membrane proteins") that are encoded on the "[plasmid] for *Yersinia* virulence" – commonly known as the pYV – cause host pathogenesis and allow the bacteria to live parasitically.

pYV

The 70kb pYV is critical to Yersiniae pathogenicity since it contains many genes known to encode virulence factors and its loss gives avirulence of all Yersiniae. A 26kb "core region" in the pYV contains the *ysc* genes which regulate the expression and secretion of Yops. Many Ysc proteins also amalgamate to form a type III secretory apparatus which secretes many Yops into the host cell cytoplasm with the assistance of the "translocation apparatus", constructed of YopB and YopD. The core region also includes *yopN*, *yopB*, *yopD*, *tyeA*, *lcrG*, and *lcrV* which also regulate Yop gene expression and help to translocate secretory Yops to the target cell. For example, YopN and TyeA are positioned as a plug on the apparatus so that only their conformational change, induced by their interaction with certain host cell membrane proteins, will cause the unblocking of the secretory pathway . Secretion is regulated in this fashion so that proteins are not expelled into the extracellular matrix and elicit an immune response. Since this pathway gives secretion selectivity, it is a virulence factor.

Effector Yops

In contrast to the *ysc* and *yop* genes listed above, the Yops that act directly on host cells to cause cytopathologic effects – "effector Yops" – are encoded by pYV genes external to this core region. The sole exception is LcrV which is also known as the "versatile Yop" for its two roles as an effector Yop and as a regulatory Yop. The combined function of these effector Yops permits the bacteria to resist internalization by immune and intestinal cells and to evade the bactericidal actions of neutrophils and macrophages. Inside the bacterium, these Yops are bound by pYV-encoded Sycs (specific Yop chaperones), which prevent premature interaction with other proteins and guide the Yops to a type III secretory apparatus. In addition to the Syc-Yop complex, Yops are also tagged for type III secretion either by the first 60nt in their corresponding mRNA transcript or by their corresponding first 20 N-terminal amino acids. LcrV, YopQ, YopE, YopT, YopH, YpkA, YopJ, YopM, and YadA are all secreted by the type III secretory pathway. LcrV inhibits neutrophil chemotaxis and cytokine production, allowing *Y. Pseudotuberculosis* to form large colonies without inducing systemic failure and, with YopQ, contributes to the translocation process by bringing YopB and YopD to the eukaryotic cell membrane for pore-formation. By causing actin filament depolymerisation, YopE, YopT, and YpkA resist endocytosis by intestinal cells and phagocytosis while giving cytotoxic changes in the host cell. YopT targets Rho GTPase, commonly named "RhoA", and uncouples it from the membrane, leaving it in an inactive RhoA-GDI (guanine nucleotide dissociation inhibitor)-bound state whereas YopE and YpkA convert Rho proteins to their inactive GDP-bound states by expressing GTPase activity. YpkA also catalyses serine autophosporylation, so it may have regulatory functions in *Yersinia* or undermine host cell immune response signal cascades since YpkA is targeted to the cytoplasmic side of the host cell membrane. YopH acts on host focal adhesion sites by dephosphorylating several phosphotyrosine residues on focal adhesion kinase (FAK) and the focal adhesion proteins paxillin and p130. Since FAK phosphorylation is involved in uptake of yersiniae as well as T cell and B cell responses to antigen-binding, YopH elicits antiphagocytic and other anti-immune effects. YopJ, which shares an operon with YpkA, "...interferes with the mitogen-activated protein (MAP) kinase activities of c-Jun N-terminal kinase (JNK), p38, and extracellular signal-regulated kinase", leading to macrophage apoptosis. In addition, YopJ inhibits TNF-α release from many cell types, possibly through an inhibitory action on NF-κB, suppressing inflammation and the immune response. By secretion through a type III pathway and localization in the nucleus by a vesicle-associated, microtubule-dependent method, YopM may alter host cell growth by binding to RSK (ribosomal S6 kinase) which regulates cell cycle regulation genes. Interestingly, YadA has lost its adhesion , opsonisation-resisting, phagocytosis-resisting, and respiratory burst-resisting functions in *Y. pseudotuberculosis* due to a frameshift mutation by a single base-pair deletion in *yadA* in comparison to *yadA* in *Y. enterocolitica*, yet it still is secreted by type III secretion. The *yop* genes, *yadA*, *ylpA*, and the *virC* operon are considered the "Yop regulon" since they are coregulated by pYV-encoded VirF. *virF* is in turn thermoregulated. At 37 degrees Celsius, chromosomally-encoded Ymo, which regulates DNA supercoiling around the *virF* gene, changes conformation, allowing for VirF expression which then up-regulates the Yop regulon.

Adhesion

Y. pseudotuberculosis adheres strongly to intestinal cells via chromosomally encoded proteins so that Yop secretion may occur, to avoid being removed by peristalsis, and to invade target host cells. A transmembrane protein, Invasin, facilitates these functions by binding to host cell αβ1 integrins. Through this binding, the integrins cluster, thereby activating FAK, and causing a corresponding reorganization of the cytoskeleton. Subsequent internalization of bound bacteria occurs when the actin-depolymerising Yops are not being expressed. The protein encoded on the "attachment invasion locus" named Ail also bestows attachment and invasive abilities upon Yersiniae while interfering with the binding of complement on the bacterial surface. To increase binding specificity, the fibrillar pH6 antigen targets bacteria to target intestinal cells only when thermoinduced.

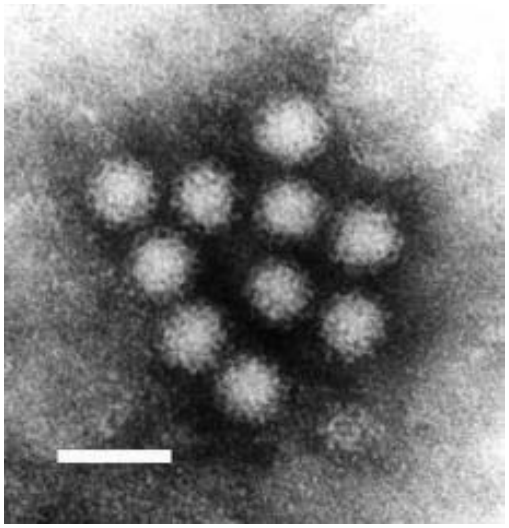
Superantigens

Certain strains of *Yersinia pseudotuberculosis* express a superantigenic exotoxin, YPM, or the *Y. pseudotuberculosis*-derived mitogen, from the chromosomal *ypm* gene. YPM specifically binds and causes the proliferation of T lymphocytes expressing the Vβ3, Vβ7, Vβ8, Vβ9, Vβ13.1, and Vβ13.2 variable regions with CD4+ T cell preference, although activation of some CD8+ T cells occurs. This T cell expansion can cause splenomegaly coupled with IL-2 and IL-4 overproduction. Since administering anti-TNF-α and anti-IFN-γ monoclonal antibodies neutralizes YPM toxicity *in vivo*, these cytokines are largely responsible for the damage caused indirectly by the exotoxin. Strains which carry the exotoxin gene are rare in Western countries where the disease, when at all apparent, manifests itself largely with minor symptoms, whereas more than 95% of strains from Far Eastern countries contain *ypm* and are correlated with Izumi fever and Kawasaki disease. Although the superantigen poses the greatest threat to host health, all virulence factors contribute to *Y. pseudotuberculosis* viability *in vivo* and define the bacterium's pathogenic characteristics. *Y. pseudotuberculosis* can live extracellularly due to its formidable mechanisms of phagocytosis and opsonisation resistance through the expression of Yops and the type III pathway; yet, by limited pYV action, it can populate host cells, especially macrophages, intracellularly to further evade immune responses and be disseminated throughout the body.

Chapter 15

Norovirus

Norovirus



Transmission electron micrograph of noroviruses. The white bar = 50 nm

Virus classification	
Group:	Group IV ((+)ssRNA)
Family:	*Caliciviridae*
Genus:	*Norovirus*

Norovirus (formerly **Norwalk agent**) is an RNA virus (taxonomic family Caliciviridae) that causes approximately 90% of epidemic non-bacterial outbreaks of gastroenteritis around the world, and may be responsible for 50% of all foodborne outbreaks of gastroenteritis in the US. Norovirus affects people of all ages. The viruses are transmitted by faecally contaminated food or water, by person-to-person contact, and via aerosolization of the virus and subsequent contamination of surfaces.

After infection, immunity to norovirus is usually incomplete and temporary. There is an inherited predisposition to infection, and individuals with blood type O are more often infected, while blood types B and AB can confer partial protection against symptomatic infection.

Outbreaks of norovirus infection often occur in closed or semi-closed communities, such as long-term care facilities, overnight camps, hospitals, prisons, dormitories, and cruise ships where the infection spreads very rapidly either by person-to-person transmission or through contaminated food. Many norovirus outbreaks have been traced to food that was handled by one infected person.

Norovirus is rapidly inactivated by either sufficient heating or by chlorine-based disinfectants, but the virus is less susceptible to alcohols and detergents as it does not have a lipid envelope.

Pathology

When a person becomes infected with norovirus, the virus begins to multiply within the small intestine. After approximately 1 to 2 days, norovirus symptoms can appear. The principal symptom is acute gastroenteritis that develops between 24 and 48 hours after exposure, and lasts for 24–60 hours. The disease is usually self-limiting, and characterized by nausea, vomiting, diarrhea, and abdominal pain; and in some cases, loss of taste. General lethargy, weakness, muscle aches, headache, and low-grade fever may occur.

Severe illness is rare: although people are frequently treated at the emergency ward, they are rarely admitted to the hospital. The number of deaths from norovirus in the US is estimated to be around 300 each year, with most of these occurring in the very young, elderly, and persons with weakened immune systems. Symptoms may become life-threatening in these groups if dehydration is ignored or not treated.

The most common genotype identified in hospitalized children was GII.4 and increased amount of virus (7.2×10^7) were shed by those children. Even though GII.4 Norovirus was the most common identified, other genotypes were identified in less proportion, including the novel GII.18-NICA.

Diagnosis

Specific diagnosis of norovirus is routinely made by polymerase chain reaction (PCR) assays or real-time PCR assays, which give results within a few hours. These assays are very sensitive and can detect concentrations as low as 10 virus particles.

Tests such as ELISA that use antibodies against a mixture of norovirus strains are available commercially but lack specificity and sensitivity.

Epidemiology, prevention and infection control

Noroviruses are transmitted directly from person to person and indirectly via contaminated fomites, water and food. They are highly contagious, with as few as one to ten virus particles being able to cause infection. Transmission occurs through ingesting

contaminated food and water and by person-to-person spread. Transmission is through fecal-oral, can be aerosolized when those stricken with the illness vomit and can be aerosolized by a toilet flush when vomit or diarrhea is present; infection can follow eating food or breathing air near an episode of vomiting, even if cleaned up. The viruses continue to be shed after symptoms have subsided and shedding can still be detected many weeks after infection.

In Guangdong province of China, The Provincial Health Department said, on December 17, 2010, it has confirmed 429 cases of norovirus infection in the November outbreak in Conghua of Guangzhou, but no one died from it.

As said previously Norovirus is extremely infectious. In one outbreak at an international scout jamboree in the Netherlands, each person with gastroenteritis infected an average of 14 people before increased hygiene measures were put in place. Even after these new measures were enacted an average ill person still infected 2.1 other people. A CDC study of eleven outbreaks in New York State lists the suspected mode of transmission as person-to-person in seven outbreaks, foodborne in two, waterborne in one, and one unknown. The source of waterborne outbreaks may include water from municipal supplies, wells, recreational lakes, swimming pools and ice machines.

Shellfish and salad ingredients are the foods most often implicated in norovirus outbreaks. Ingestion of shellfish that have not been sufficiently heated poses a high risk for norovirus infection. Foods other than shellfish may be contaminated by infected food handlers.

Hand washing is an effective method to reduce the spread of norovirus pathogens. Sanitizing of surfaces where the norovirus may be present is recommended. Alcohol rubs are *not* very effective at dealing with Norovirus as it does not have a lipid viral envelope.

In health-care environments, the prevention of nosocomial infections involves routine and terminal cleaning. Nonflammable alcohol vapor in CO_2 systems are used in health care environments where medical electronics would be adversely affected by aerosolized chlorine or other caustic compounds.

Ligocyte announced in 2007 that it was working on a vaccine and had started phase 1 trials.

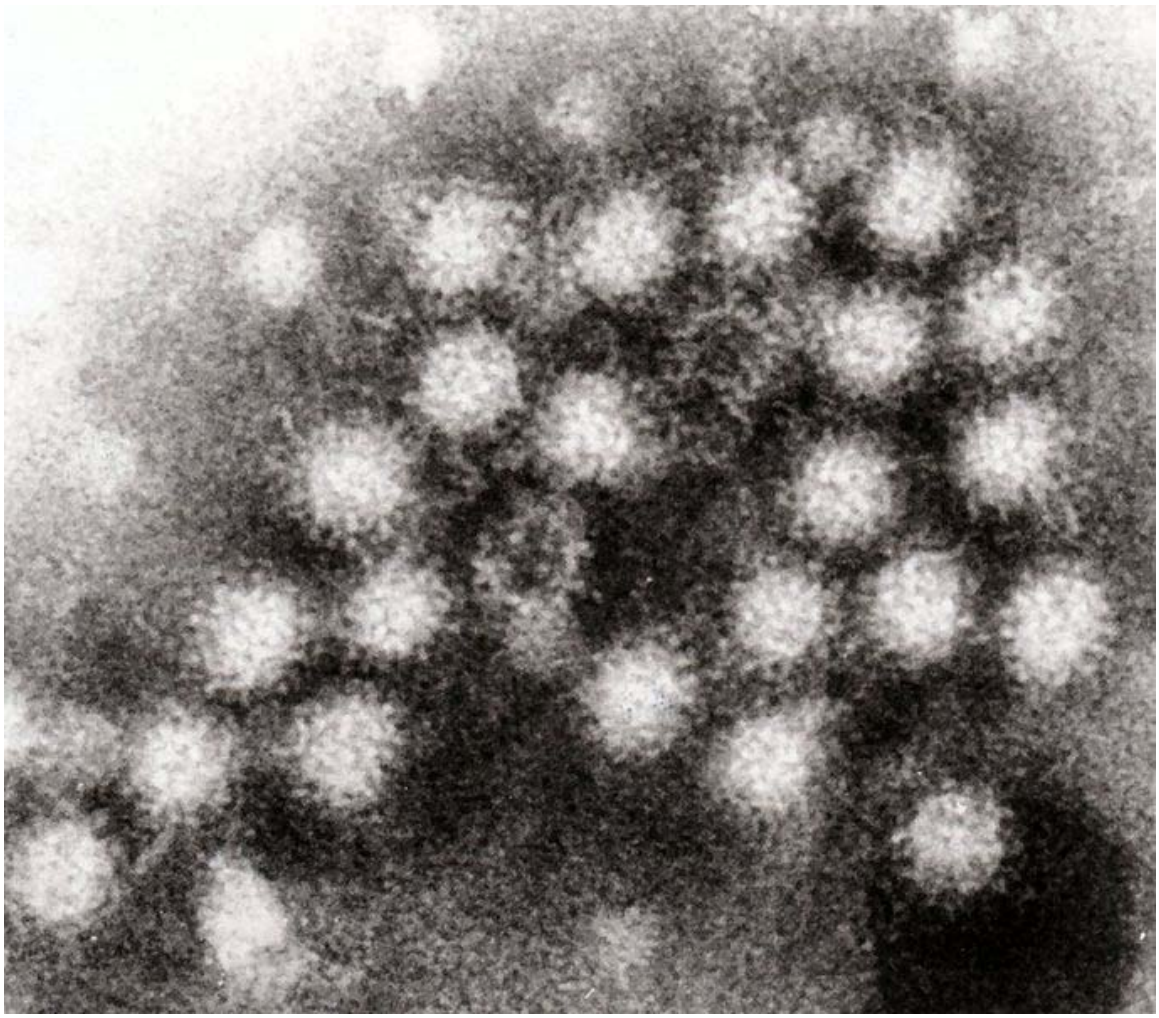
Epidemiological data from developing countries about the importance of norovirus in pediatric diarrhea is limited. Recently, in Nicaragua, it has been observed that norovirus is responsible for 11% of the diarrhea cases occurring in children <5 years of age at community level and 15% of the moderate to severe cases requiring intravenous re-hydration.

Detection in foods

Routine protocols to detect norovirus (norovirus RNA) in clams and oysters by RT-PCR (reverse transcription polymerase chain reaction) are being employed by governmental laboratories such as the FDA in the USA. However, routine methods to detect the virus on other food items are not readily available due to the variable nature of different food items affecting concentration and extraction of the virus and presence of factors that make PCR (polymerase chain reaction) analysis techniques ineffective.

Microbiology

Classification



Transmission electron micrograph of Norovirus particles in faeces

Noroviruses (NoV) are a genetically diverse group of single stranded RNA, nonenveloped viruses belonging to the Caliciviridae family. According to the

International Committee on Taxonomy of Virus, the genus Norovirus has one species, which is called "Norwalk virus" and assigned the abbreviation "NV". Serotypes, strains and isolates include:

- Norwalk virus [M87661] (Hu/NLV/NV/1968/US)
- Hawaii virus [U07611] (Hu/NLV/HV/1971/US)
- Snow Mountain virus [L23831] (Hu/NLV/SMV/1976/US)
- Mexico virus [U22498] (Hu/NLV/MX/1989/MX)
- Desert Shield virus [U04469] (Hu/NLV/DSV395/1990/SR)
- Southampton virus [L07418] (Hu/NLV/SHV/1991/UK)
- Lordsdale virus [X86557] (Hu/NLV/LD/1993/UK)

Noroviruses commonly isolated in cases of acute gastroenteritis belong to two genogroups: genogroup I (GI) includes Norwalk virus, Desert Shield virus and Southampton virus and II (GII), which includes Bristol virus, Lordsdale virus, Toronto virus, Mexico virus, Hawaii virus and Snow Mountain virus.

Noroviruses can genetically be classified into 5 different genogroups (GI, GII, GIII, GIV, and GV), which can be further divided into different genetic clusters or genotypes. For example genogroup II, the most prevalent human genogroup, presently contains 19 genotypes. Genogroups I, II and IV infect humans, whereas genogroup III infects bovine species and genogroup V has recently been isolated in mice.

Most noroviruses that infect humans belong to genogroup GI and GII. Noroviruses from Genogroup II, genotype 4 (abbreviated as GII.4) account for the majority of adult outbreaks of gastroenteritis and often sweep across the globe. Recent examples include US95/96-US strain, associated with global outbreaks in the mid- to late-90s, Farmington Hills virus associated with outbreaks in Europe and the United States in 2002 and in 2004 Hunter virus was associated with outbreaks in Europe, Japan and Australasia. In 2006 there was another large increase in NoV infection around the globe. Two new GII.4 variants caused around 80% of those Norovirus associated outbreaks and they have been termed 2006a and 2006b. Recent reports have shown a link between blood group and susceptibility to infection by norovirus.

A 2008 study suggests that the protein MDA-5 may be the primary immune sensor that detects the presence of noroviruses in the body. Interestingly, some people have common variations of the MDA-5 gene that could make them more susceptible to norovirus infection.

A 2010 study suggested that a specific genetic version of Norovirus (which would not be distinguishable from other types of the virus using standard viral antibody tests) interacts with a specific mutation in the ATG16L1 gene to help trigger symptomatic Crohn's Disease in mice that have been subjected to a chemical that causes intestinal injury similar to the process in humans (there are other similar ways for such diseases to happen like this, and this study in itself does not prove that Norovirus causes Crohn's in humans).

Virus structure

Noroviruses contain a positive-sense RNA genome of approximately 7.5 kbp, encoding a major structural protein (VP1) of about 58~60 kDa and a minor capsid protein (VP2). The virus particles demonstrate an amorphous surface structure when visualized using electron microscopy and are between 27-38 nm in size.

History

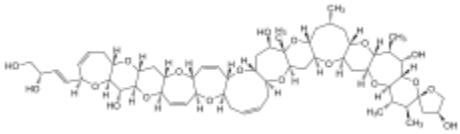
The norovirus was originally named the *Norwalk agent* after Norwalk, Ohio, USA, where an outbreak of acute gastroenteritis occurred among children at Bronson Elementary School in November 1968. The name was shortened to *norovirus* after being identified in a number of outbreaks on cruise ships and receiving attention throughout the USA. In 1972, electron microscopy on stored human stool samples identified a virus, which was given the name Norwalk virus. Numerous outbreaks with similar symptoms have been reported since. The cloning and sequencing of the Norwalk virus genome showed that these viruses have a genomic organization consistent with viruses belonging to the family Caliciviridae. The name norovirus (*Norovirus* for the genus) was approved by the International Committee on Taxonomy of Viruses in 2002.

Common names of the illness caused by noroviruses are winter vomiting disease, viral gastroenteritis, and acute non-bacterial gastroenteritis, also colloquially known as "stomach flu"—a broad name that refers to gastric inflammation caused by a range of viruses and bacteria.

Chapter 16

Ciguatera

Ciguatera fish poisoning



Chemical structure of the ciguatoxin-CTX1B

ICD-10	T61.0
ICD-9	988.0
DiseasesDB	31122
MedlinePlus	002851
eMedicine	emerg/100 ped/403

Ciguatera is a foodborne illness caused by eating certain reef fishes whose flesh is contaminated with toxins originally produced by dinoflagellates such as *Gambierdiscus toxicus* which lives in tropical and subtropical waters. These dinoflagellates adhere to coral, algae and seaweed, where they are eaten by herbivorous fish who in turn are eaten by larger carnivorous fish. In this way the toxins move up the foodchain and bioaccumulate. *Gambierdiscus toxicus* is the primary dinoflagellate responsible for the production of a number of similar toxins that cause ciguatera. These toxins include ciguatoxin, maitotoxin, scaritoxin and palytoxin. Predator species near the top of the food chain in tropical and subtropical waters, such as barracudas, snapper, moray eels, parrotfishes, groupers, triggerfishes and amberjacks, are most likely to cause ciguatera poisoning, although many other species cause occasional outbreaks of toxicity. Ciguatoxin is very heat-resistant, so ciguatoxin-laden fish cannot be detoxified by conventional cooking.

Researchers suggest that ciguatera outbreaks caused by cooling climatic conditions propelled the migratory voyages of Polynesians between 1000 and 1400.

Symptoms

Hallmark symptoms of ciguatera in Humans include gastrointestinal and neurological effects. Gastrointestinal symptoms include nausea, vomiting, and diarrhea, usually followed by neurological symptoms such as headaches, muscle aches, paresthesia, numbness, ataxia, and hallucinations. Severe cases of ciguatera can also result in cold allodynia, which is a burning sensation on contact with cold (commonly incorrectly referred to as reversal of hot/cold temperature sensation). Doctors are often at a loss to explain these symptoms and ciguatera poisoning is frequently misdiagnosed as multiple sclerosis.

Dyspareunia and other ciguatera symptoms have developed in otherwise healthy males and females following sexual intercourse with partners suffering ciguatera poisoning, signifying that the toxin may be sexually transmitted. Diarrhea and facial rashes have been reported in breastfed infants of poisoned mothers, it is likely that ciguatera toxins migrate into breast milk.

The symptoms can last from weeks to years, and in extreme cases as long as 20 years, often leading to long-term disability. Most people do recover slowly over time. Often patients recover, but symptoms then reappear. Such relapses can be triggered by consumption of nuts, alcohol, fish or fish-containing products, chicken or eggs, or by exposure to fumes such as those of bleach and other chemicals. Exercise is also a possible trigger. Filipino and Chinese people may possibly be more susceptible.

Detection methods

Scientific detection

Currently, multiple laboratory methods are available to detect ciguatoxins, including liquid chromatography-mass spectrometry (LCMS), receptor binding assays (RBA), and neuroblastoma assays (N2A). Although testing is possible, in most cases liquid chromatography-mass spectrometry is insufficient to detect clinically relevant concentrations of ciguatoxin in crude extracts of fish.

Folk detection

In Northern Australia, where ciguatera is a common problem, two different folk science methods are widely believed to detect whether fish harbor significant ciguatoxin. The first method is that flies will not land on contaminated fish. The second is that cats display symptoms after eating contaminated fish. A third, less common testing method involves putting a silver coin under the scales of the suspect fish. Only if the coin turns black, is it contaminated. It is not known whether any of these tests produce accurate results, although the cat most likely does.

Folk remedies

Various Caribbean naturopathic and ritualistic treatments originated in Cuba and nearby islands. The most common old-time remedy involves bed rest subsequent to a guanabana juice enema. Other folk treatments range from directly porting and bleeding the gastrointestinal tract to "cleansing" the diseased with a dove during a Santeria ritual. In Puerto Rico, natives drink a tea made from mangrove buttons, purportedly high in B Vitamins, to flush the toxic symptoms from the system. The efficacy of these treatments has never been studied or substantiated.

An account of ciguatera poisoning from a linguistics researcher living on Malakula island, Vanuatu, indicates the local treatment: "We had to go with what local people told us: avoid salt and any seafood. Eat sugary foods. And they gave us a tea made from the roots of ferns growing on tree trunks. I don't know if any of that helped, but after a few weeks, the symptoms faded away."

Treatment

There is no effective treatment or antidote for ciguatera poisoning. The mainstay of treatment is supportive care. There is some evidence that calcium channel blocker type drugs such as Nifedipine and Verapamil are effective in treating some of the symptoms that remain after the initial sickness passes, such as poor circulation and shooting pains through the chest. These symptoms are due to the cramping of arterial walls caused by maitotoxin Ciguatoxin lowers the threshold for opening voltage-gated sodium channels in synapses of the nervous system. Opening a sodium channel causes depolarization, which could sequentially cause paralysis, heart contraction, and changing the senses of hearing and cold. Nifedipine is a calcium channel blocker. Some medications such as Amitriptyline may reduce some symptoms, such as fatigue and paresthesia, although benefit does not occur in every case. Steroids and vitamin supplements support the body's recovery rather than directly reducing toxin effects.

Mannitol was once used for poisoning after one study reported symptom reversal. Followup studies in animals and case reports in humans also found benefit from mannitol. However, a randomized, double-blind clinical trial found no difference between mannitol and normal saline, and based on this result, mannitol is no longer recommended.

Epidemiology

Due to the limited habitats of ciguatoxin-producing microorganisms, ciguatera is common in only subtropical and tropical waters, particularly the Pacific and Caribbean, and usually is associated with fish caught in tropical reef waters. Ciguatoxin is found in over 400 species of reef fish. Avoiding consumption of all reef fish (any fish living in warm tropical waters) is the only sure way to avoid exposure. Imported fish served in restaurants may contain the toxin and to produce illness which often goes unexplained by

physicians unfamiliar with the symptoms of a tropical toxin. Ciguatoxin can also occur in farm-raised salmon.

In 2007, ten people in St. Louis, Missouri developed the disease after eating imported fish.

In February 2008, the U.S. Food and Drug Administration (FDA) traced several outbreaks to the Flower Garden Banks National Marine Sanctuary in the northern Gulf of Mexico, near the Texas-Louisiana shoreline. The FDA advised seafood processors that ciguatera poisoning was "reasonably likely" to occur from eating several species of fish caught as far as 50 miles (80 km) from the sanctuary.

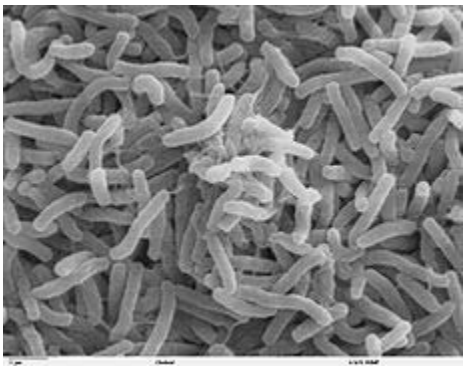
History

Ciguatera was first described by one of the surgeon's mates, John Anderson, on the crew of HMS Resolution in 1774.

Chapter 17

Cholera

Cholera

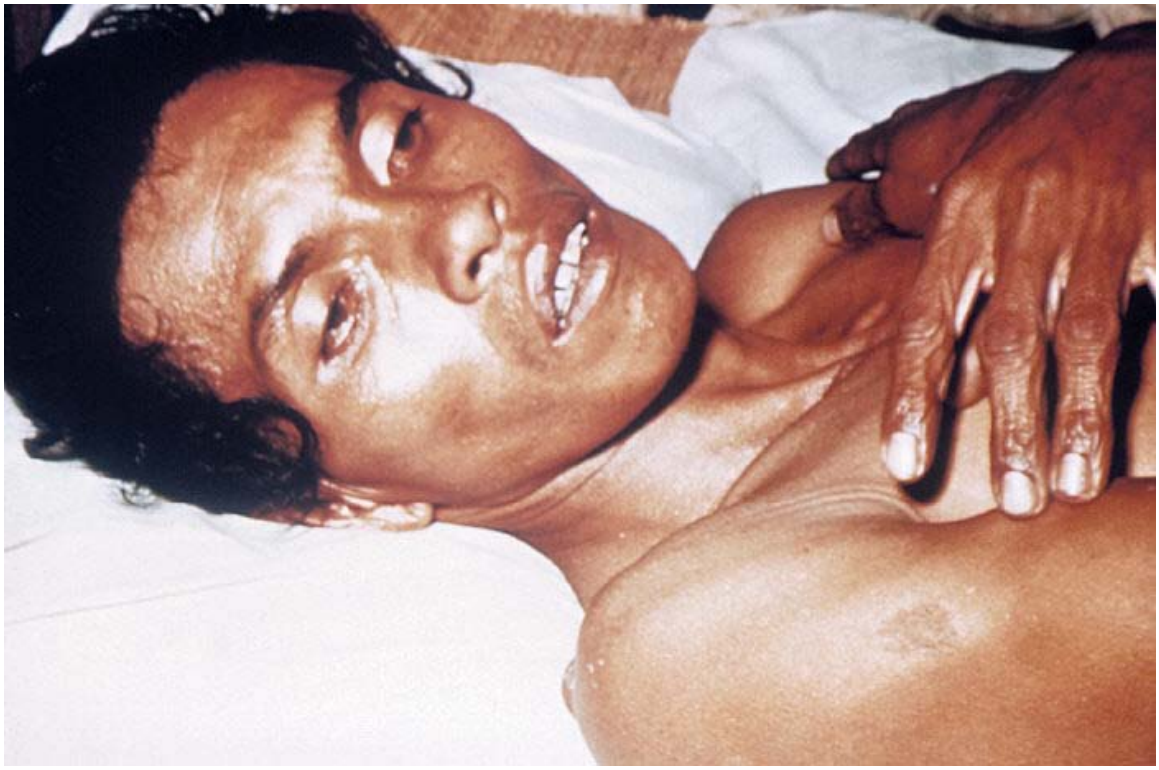


Scanning electron microscope image of *Vibrio cholerae*

ICD-10	A00.
ICD-9	001
DiseasesDB	29089
MedlinePlus	000303
eMedicine	med/351
MeSH	D002771

Cholera is an infection of the small intestine that is caused by the bacterium *Vibrio cholerae*. The main symptoms are profuse watery diarrhea and vomiting. Transmission is primarily through consuming contaminated drinking water or food. The severity of the diarrhea and vomiting can lead to rapid dehydration and electrolyte imbalance. Primary treatment is with oral rehydration solution and if these are not tolerated, intravenous fluids. Antibiotics are beneficial in those with severe disease. Worldwide it affects 3-5 million people and causes 100,000-130,000 deaths a year as of 2010. Cholera was one of the earliest infections to be studied by epidemiological methods.

Signs and symptoms



A person with severe dehydration due to cholera. Note the sunken eyes and decreased skin turgor which produces wrinkled hands

The primary symptoms of cholera are profuse painless diarrhea and vomiting of clear fluid. These symptoms usually start suddenly, one to five days after ingestion of the bacteria. The diarrhea is frequently described as "rice water" in nature and may have a fishy odor. An untreated person with cholera may produce 10-20 liters of diarrhea a day with fatal results. For every symptomatic person there are 3 to 100 people who get the infection but remain asymptomatic.

If the severe diarrhea and vomiting are not aggressively treated it can, within hours, result in life-threatening dehydration and electrolyte imbalances. The typical symptoms of dehydration include low blood pressure, poor skin turgor (wrinkled hands), sunken eyes, and a rapid pulse.

Cause

Cholera is caused by eating contaminated food. Transmission is primarily due to the fecal contamination of food and water due to poor sanitation. This bacterium can, however, live naturally in any environment.

Susceptibility

About one hundred million bacteria must typically be ingested to cause cholera in a normal healthy adult. This dose, however, is less in those with lower gastric acidity (for instance those using proton pump inhibitors). Children are also more susceptible with two to four year olds having the highest rates of infection.

It has been said that cystic fibrosis genetic mutation in humans has maintained a selective advantage: heterozygous carriers of the mutation (who are thus not affected by cystic fibrosis) are more resistant to *V. cholerae* infections. In this model, the genetic deficiency in the cystic fibrosis transmembrane conductance regulator channel proteins interferes with bacteria binding to the gastrointestinal epithelium, thus reducing the effects of an infection.

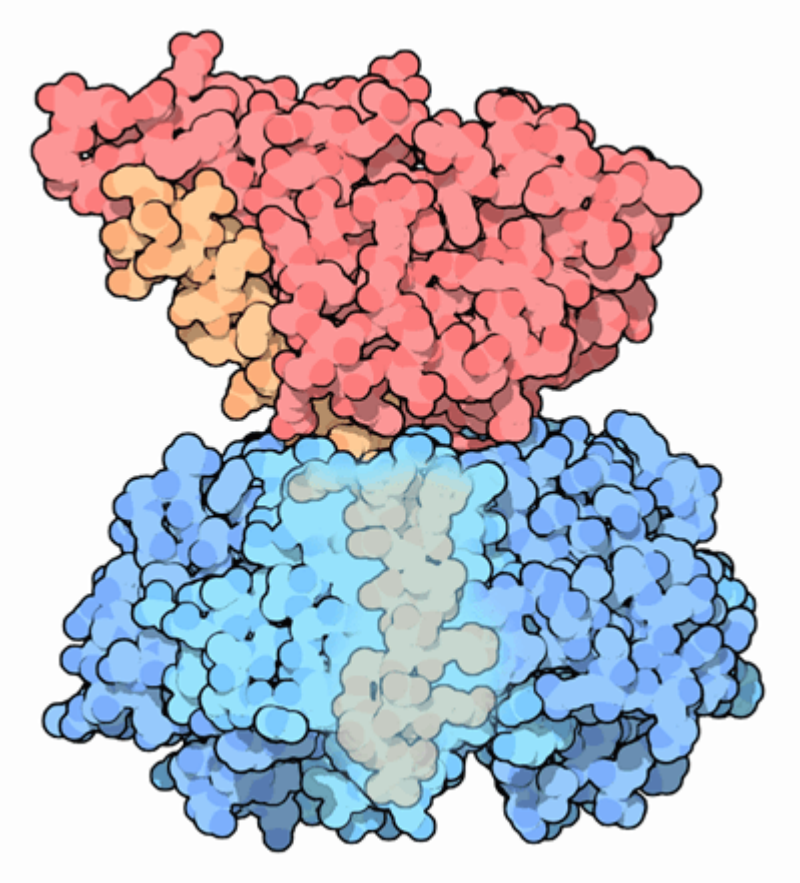
Individuals' susceptibility to cholera is affected by their blood type, with those with type O blood being the most susceptible.

Transmission

Cholera is typically transmitted by either contaminated food or water. In the developed world, seafood is the usual cause, while in the developing world it is more often water. Cholera has been found in only two other animal populations: shellfish and plankton.

People infected with cholera often have diarrhea, and if this highly liquid stool, colloquially referred to as "rice-water," contaminates water used by others, disease transmission may occur. The source of the contamination is typically other cholera sufferers when their untreated diarrheal discharge is allowed to get into waterways or into groundwater or drinking water supplies. Drinking any infected water and eating any foods washed in the water, as well as shellfish living in the affected waterway, can cause a person to contract an infection. Cholera is rarely spread directly from person to person. Both toxic and nontoxic strains exist. Nontoxic strains can acquire toxicity through a lysogenic bacteriophage. Coastal cholera outbreaks typically follow zooplankton blooms, thus making cholera a zoonotic disease.

Mechanism



Cholera toxin: The delivery region (blue) binds membrane carbohydrates to get into cells. The toxic part (red) is activated inside the cell (PDB code: 1xtc).

Most bacteria, when consumed, do not survive the acidic conditions of the human stomach. The few bacteria that do survive conserve their energy and stored nutrients during the passage through the stomach by shutting down much protein production. When the surviving bacteria exit the stomach and reach the small intestine, they need to propel themselves through the thick mucus that lines the small intestine to get to the intestinal walls, where they can thrive. *V. cholerae* bacteria start up production of the hollow cylindrical protein flagellin to make flagella, the cork-screw helical fibers they rotate to propel themselves through the mucus of the small intestine.

Once the cholera bacteria reach the intestinal wall, they no longer need the flagella propellers to move. The bacteria stop producing the protein flagellin, thus again conserving energy and nutrients by changing the mix of proteins which they manufacture in response to the changed chemical surroundings. On reaching the intestinal wall, *V. cholerae* start producing the toxic proteins that give the infected person a watery diarrhea. This carries the multiplying new generations of *V. cholerae* bacteria out into the drinking water of the next host if proper sanitation measures are not in place.

The cholera toxin (CTX or CT) is an oligomeric complex made up of six protein subunits: a single copy of the A subunit (part A), and five copies of the B subunit (part B), connected by a disulfide bond. The five B subunits form a five-membered ring that binds to GM1 gangliosides on the surface of the intestinal epithelium cells. The A1 portion of the A subunit is an enzyme that ADP-ribosylates G proteins, while the A2 chain fits into the central pore of the B subunit ring. Upon binding, the complex is taken into the cell via receptor-mediated endocytosis. Once inside the cell, the disulfide bond is reduced, and the A1 subunit is freed to bind with a human partner protein called ADP-ribosylation factor 6 (Arf6). Binding exposes its active site, allowing it to permanently ribosylate the Gs alpha subunit of the heterotrimeric G protein. This results in constitutive cAMP production, which in turn leads to secretion of H_2O, Na^+, K^+, Cl^-, and HCO_3^- into the lumen of the small intestine and rapid dehydration. The gene encoding the cholera toxin is introduced into *V. cholerae* by horizontal gene transfer. Virulent strains of *V. cholerae* carry a variant of lysogenic bacteriophage called CTXf or CTXφ.

Microbiologists have studied the genetic mechanisms by which the *V. cholerae* bacteria turn off the production of some proteins and turn on the production of other proteins as they respond to the series of chemical environments they encounter, passing through the stomach, through the mucous layer of the small intestine, and on to the intestinal wall. Of particular interest have been the genetic mechanisms by which cholera bacteria turn on the protein production of the toxins that interact with host cell mechanisms to pump chloride ions into the small intestine, creating an ionic pressure which prevents sodium ions from entering the cell. The chloride and sodium ions create a salt-water environment in the small intestines, which through osmosis can pull up to six liters of water per day through the intestinal cells, creating the massive amounts of diarrhea. The host can become rapidly dehydrated if an appropriate mixture of dilute salt water and sugar is not taken to replace the blood's water and salts lost in the diarrhea.

By inserting separate, successive sections of *V. cholerae* DNA into the DNA of other bacteria, such as *E. coli* that would not naturally produce the protein toxins, researchers have investigated the mechanisms by which *V. cholerae* responds to the changing chemical environments of the stomach, mucous layers, and intestinal wall. Researchers have discovered there is a complex cascade of regulatory proteins that control expression of *V. cholerae* virulence determinants. In responding to the chemical environment at the intestinal wall, the *V. cholerae* bacteria produce the TcpP/TcpH proteins, which, together with the ToxR/ToxS proteins, activate the expression of the ToxT regulatory protein. ToxT then directly activates expression of virulence genes that produce the toxins, causing diarrhea in the infected person and allowing the bacteria to colonize the intestine. Current research aims at discovering "the signal that makes the cholera bacteria stop swimming and start to colonize (that is, adhere to the cells of) the small intestine."

Genetic structure

Amplified fragment length polymorphism (AFLP) fingerprinting of the pandemic isolates of *Vibrio cholerae* has revealed variation in the genetic structure. Two clusters have been identified: Cluster I and Cluster II. For the most part, Cluster I consists of strains from the

1960s and 1970s, while Cluster II largely contains strains from the 1980s and 1990s, based on the change in the clone structure. This grouping of strains is best seen in the strains from the African continent.

Diagnosis

In epidemic situations, a clinical diagnosis may be made by taking a history and doing a brief examination. Treatment is usually started without or before confirmation by laboratory analysis.

A rapid dip-stick test is available to determine the presence of *V. cholerae*. In those that test positive, further testing should be done to determine antibiotic resistance.

Stool and swab samples collected in the acute stage of the disease, before antibiotics have been administered, are the most useful specimens for laboratory diagnosis. If an epidemic of cholera is suspected, the most common causative agent is *Vibrio cholerae* O1. If *V. cholerae* serogroup O1 is not isolated, the laboratory should test for *V. cholerae* O139. However, if neither of these organisms is isolated, it is necessary to send stool specimens to a reference laboratory. Infection with *V. cholerae* O139 should be reported and handled in the same manner as that caused by *V. cholerae* O1. The associated diarrheal illness should be referred to as cholera and must be reported in the United States.

A number of special media have been employed for the cultivation for cholera vibrios. They are classified as follows:

Enrichment media

1. *Alkaline peptone water* at pH 8.6
2. *Monsur's taurocholate tellurite peptone water* at pH 9.2

Plating media

1. *Alkaline bile salt agar (BSA)*: The colonies are very similar to those on nutrient agar.
2. *Monsur's gelatin Tauro cholate trypticase tellurite agar (GTTA) medium*: Cholera vibrios produce small translucent colonies with a greyish black center.
3. *TCBS medium*: This the mostly widely used medium; it contains thiosulphate, citrate, bile salts and sucrose. Cholera vibrios produce flat 2–3 mm in diameter, yellow nucleated colonies.

Direct microscopy of stool is not recommended, as it is unreliable. Microscopy is preferred only after enrichment, as this process reveals the characteristic motility of *Vibrio* and its inhibition by appropriate antisera. Diagnosis can be confirmed, as well, as serotyping done by agglutination with specific sera.

Prevention

Cholera hospital in Dhaka, showing typical *cholera beds*

Although cholera may be life-threatening, prevention of the disease is normally straightforward if proper sanitation practices are followed. In developed countries, due to nearly universal advanced water treatment and sanitation practices, cholera is no longer a major health threat. The last major outbreak of cholera in the United States occurred in 1910-1911. Effective sanitation practices, if instituted and adhered to in time, are usually sufficient to stop an epidemic. There are several points along the cholera transmission path at which its spread may be halted:

- Sterilization: Proper disposal and treatment of infected fecal waste water produced by cholera victims and all contaminated materials (e.g. clothing, bedding, etc.) is essential. All materials that come in contact with cholera patients should be sterilized by washing in hot water, using chlorine bleach if possible. Hands that touch cholera patients or their clothing, bedding, etc., should be thoroughly cleaned and disinfected with chlorinated water or other effective antimicrobial agents.

- Sewage: antibacterial treatment of general sewage by chlorine, ozone, ultraviolet light or other effective treatment before it enters the waterways or underground water supplies helps prevent undiagnosed patients from inadvertently spreading the disease.
- Sources: Warnings about possible cholera contamination should be posted around contaminated water sources with directions on how to decontaminate the water (boiling, chlorination etc.) for possible use.
- Water purification: All water used for drinking, washing, or cooking should be sterilized by either boiling, chlorination, ozone water treatment, ultraviolet light sterilization (e.g. by solar water disinfection), or antimicrobial filtration in any area where cholera may be present. Chlorination and boiling are often the least expensive and most effective means of halting transmission. Cloth filters, though very basic, have significantly reduced the occurrence of cholera when used in poor villages in Bangladesh that rely on untreated surface water. Better antimicrobial filters, like those present in advanced individual water treatment hiking kits, are most effective. Public health education and adherence to appropriate sanitation practices are of primary importance to help prevent and control transmission of cholera and other diseases.

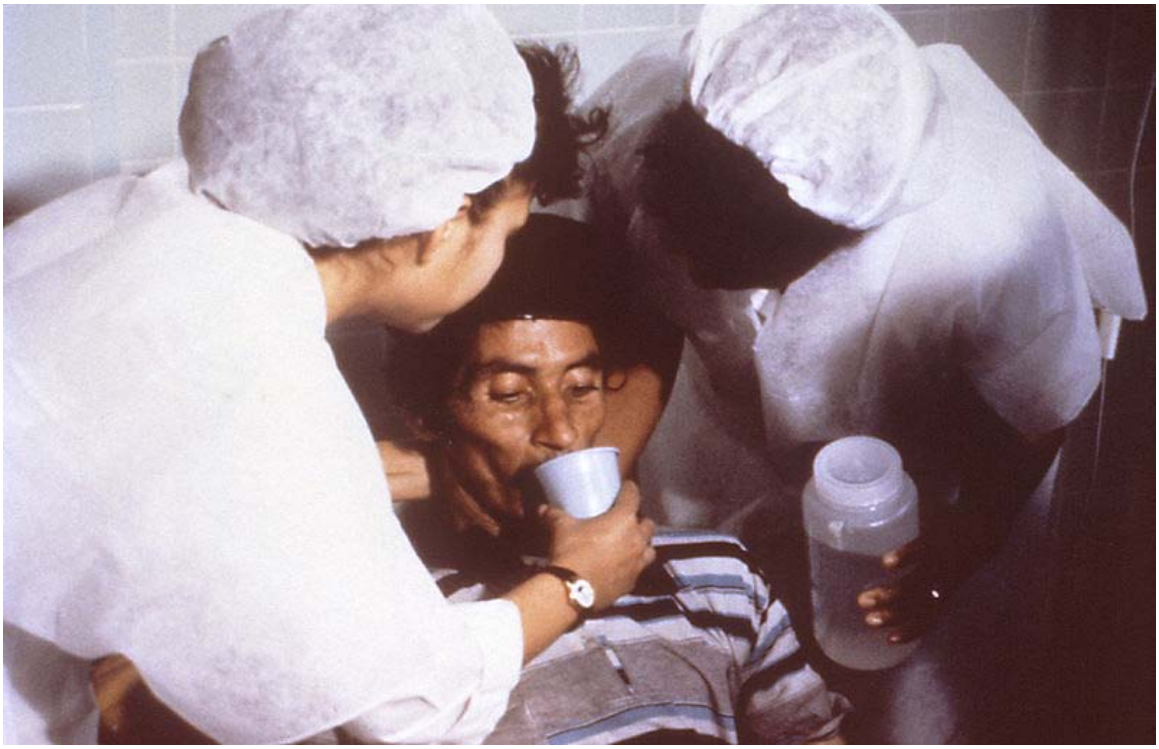
Surveillance

Surveillance and prompt reporting allow for containing cholera epidemics rapidly. Cholera exists as a seasonal disease in many endemic countries, occurring annually mostly during rainy seasons. Surveillance systems can provide early alerts to outbreaks, therefore leading to coordinated response and assist in preparation of preparedness plans. Efficient surveillance systems can also improve the risk assessment for potential cholera outbreaks. Understanding the seasonality and location of outbreaks provide guidance for improving cholera control activities for the most vulnerable. For prevention to be effective it is important that cases are reported to national health authorities.

Vaccine

A number of safe and effective oral vaccines for cholera are available. Dukoral, an orally administered, inactivated whole cell vaccine, has an efficacy of 85%, with minimal side effects. It is available in over 60 countries. However, it is not currently recommended by the Centers for Disease Control and Prevention (CDC) for most people traveling from the United States to the third world. One injectable vaccine was found to be effective for two to three years. However, as of 2010, it has limited availability. Work is under way to investigate the role of mass vaccination. The World Health Organization (WHO) recommends immunization of high risk groups, such as children and people with HIV, in countries where this disease is endemic. If people are immunized broadly, herd immunity results, with a decrease in the amount of contamination in the environment.

Treatment



Cholera patient being treated by medical staff in 1992

Fluids

In most cases, cholera can be successfully treated with oral rehydration therapy (ORT),which is highly effective, safe, and simple to administer. Rice-based solutions are preferred to glucose-based ones due to greater efficacy. In severe cases with significant dehydration, intravenous rehydration may be necessary. Ringer's lactate is the preferred solution. Large volumes and continued replacement until diarrhea has subsided may be needed. Ten percent of a person's body weight in fluid may need to be given in the first two to four hours.

If commercially produced oral rehydration solutions are too expensive or difficult to obtain, solutions can be made. One such recipe calls for 1 liter of boiled water, 1 teaspoon of salt, 8 teaspoons of sugar, and added mashed banana for potassium and to improve taste.

Electrolytes

As there frequently is initially acidosis, the potassium level may be normal, even though large losses have occurred. As the dehydration is corrected, potassium levels may decrease rapidly, and thus need to be replaced.

Antibiotics

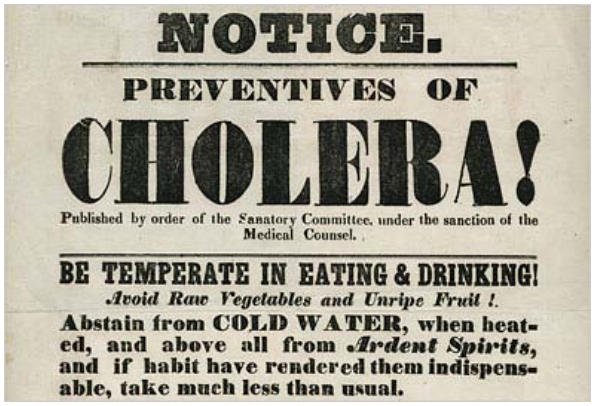
Antibiotic treatments for one to three days shorten the course of the disease and reduce the severity of the symptoms. People will recover without them, however, if sufficient hydration is maintained. Doxycycline is typically used first line, although some strains of *V. cholerae* have shown resistance. Testing for resistance during an outbreak can help determine appropriate future choices. Other antibiotics that have been proven effective include cotrimoxazole, erythromycin, tetracycline, chloramphenicol, and furazolidone. Fluoroquinolones, such as norfloxacin, also may be used, but resistance has been reported.

In many areas of the world, antibiotic resistance is increasing. In Bangladesh, for example, most cases are resistant to tetracycline, trimethoprim-sulfamethoxazole, and erythromycin. Rapid diagnostic assay methods are available for the identification of multiple drug-resistant cases. New generation antimicrobials have been discovered which are effective against in *in vitro* studies.

Prognosis

If people with cholera are treated quickly and properly, the mortality rate is less than 1%; however, with untreated cholera, the mortality rate rises to 50–60%. For certain genetic strains of cholera, such as the one present during the 2010 epidemic in Haiti and the 2004 outbreak in India, death can occur within two hours of the first sign of symptoms.

Epidemiology


NOTICE.

PREVENTIVES OF

CHOLERA!

Published by order of the Sanatory Committee, under the sanction of the Medical Counsel.

BE TEMPERATE IN EATING & DRINKING!

Avoid Raw Vegetables and Unripe Fruit !.

Abstain from COLD WATER, when heated, and above all from *Ardent Spirits*, and if habit have rendered them indispensable, take much less than usual.

Hand bill from the New York City Board of Health, 1832. The outdated public health advice demonstrates the lack of understanding of the disease and its actual causative factors.

It is estimated that cholera affects 3-5 million people worldwide, and causes 100,000-130,000 deaths a year as of 2010. This occurs mainly in the developing world. In the early 1980s, death rates are believed to have been greater than 3 million a year. It is

difficult to calculate exact numbers of cases, as many go unreported due to concerns that an outbreak may have a negative impact on the tourism of a country. Cholera remains both epidemic and endemic in many areas of the world.

Although much is known about the mechanisms behind the spread of cholera, this has not led to a full understanding of what makes cholera outbreaks happen some places and not others. Lack of treatment of human feces and lack of treatment of drinking water greatly facilitate its spread, but bodies of water can serve as a reservoir, and seafood shipped long distances can spread the disease. Cholera was not known in the Americas for most of the 20th century, but it reappeared towards the end of that century and seems likely to persist.

History

The word cholera is from Greek: χολέρα *kholera* from χολή *kholē* "bile". Cholera likely has its origins in the Indian subcontinent; it has been prevalent in the Ganges delta since ancient times. The disease first spread by trade routes (land and sea) to Russia in 1817, then to Western Europe, and from Europe to North America. There have been seven cholera pandemics in the past 200 years, with the seventh originating in Indonesia in 1961.

From a local disease, cholera became one of the most widespread and deadly diseases of the 19th century, killing an estimated tens of millions of people. In Russia alone, between 1847 and 1851, more than one million people had perished of the disease. It killed 150,000 Americans during the second pandemic. Between 1900 and 1920, perhaps eight million people died of cholera in India.

Cholera became the first reportable disease in the United States due to the significant effects it had on health. John Snow, in 1854, was the first to identify the importance of contaminated water in its cause. Cholera is now no longer considered a pressing health threat in Europe and North America due to filtering and chlorination of water supplies, but still heavily affects populations in developing countries.

In the past, people traveling in ships would hang a yellow quarantine flag if one or more of the crew members suffered from cholera. Passengers from boats with a yellow flag hung would not be allowed to disembark at any harbor for an extended period, typically 30 to 40 days. In modern international maritime signal flags, the quarantine flag is yellow and black.

Cholera morbus

The term *cholera morbus* was used in the 19th and early 20th centuries to describe both nonepidemic cholera and other gastrointestinal diseases (sometimes epidemic) that resembled cholera. The term is not in current use, but is found in many older references. The other diseases are now known collectively as gastroenteritis.

Research

The Russian-born bacteriologist Waldemar Haffkine developed the first cholera vaccine around 1900. The bacterium had been originally isolated forty five years earlier (1855) by Italian anatomist Filippo Pacini, but its exact nature and his results were not widely known.

One of the major contributions to fighting cholera was made by the physician and pioneer medical scientist John Snow (1813–1858), who in 1854 found a link between cholera and contaminated drinking water. Dr. Snow proposed a microbial origin for epidemic cholera in 1849. In his major "state of the art" review of 1855, he proposed a substantially complete and correct model for the etiology of the disease. In two pioneering epidemiological field studies, he was able to demonstrate human sewage contamination was the most probable disease vector in two major epidemics in London in 1854. His model was not immediately accepted, but it was seen to be the more plausible, as medical microbiology developed over the next thirty years or so.

Cities in developed nations made massive investment in clean water supply and well-separated sewage treatment infrastructures between the mid-1850s and the 1900s. This eliminated the threat of cholera epidemics from the major developed cities in the world. In 1885, Robert Koch identified *V. cholerae* with a microscope as the bacillus causing the disease..

Cholera has been a laboratory for the study of evolution of virulence. The province of Bengal in British India was partitioned into West Bengal and East Pakistan in 1947. Prior to partition, both regions had cholera pathogens with similar characteristics. After 1947, India made more progress on public health than East Pakistan (now Bangladesh). As a consequence, the strains of the pathogen that succeeded in India had a greater incentive in the longevity of the host. They have become less virulent than the strains prevailing in Bangladesh. These draw upon the resources of the host population and rapidly kill many victims.

More recently, in 2002, Alam, et al., studied stool samples from patients at the International Centre for Diarrhoeal Disease (ICDDR) in Dhaka, Bangladesh. From the various experiments they conducted, the researchers found a correlation between the passage of *V. cholerae* through the human digestive system and an increased infectivity state. Furthermore, the researchers found the bacterium creates a hyperinfected state where genes that control biosynthesis of amino acids, iron uptake systems, and formation of periplasmic nitrate reductase complexes were induced just before defecation. These induced characteristics allow the cholera vibrios to survive in the "rice water" stools, an environment of limited oxygen and iron, of patients with a cholera infection.

Notable cases

- Tchaikovsky's death has traditionally been attributed to cholera, most probably contracted through drinking contaminated water several days earlier. Since the

water was not boiled and cholera was affecting St. Petersburg, such a connection was quite plausible ...." Tchaikovsky's mother died of cholera, and his father became sick with cholera at this time but made a full recovery. But some scholars, including English musicologist and Tchaikovsky authority David Brown and biographer Anthony Holden, have theorized that his death was a suicide.

Other famous people believed to have died of cholera include:

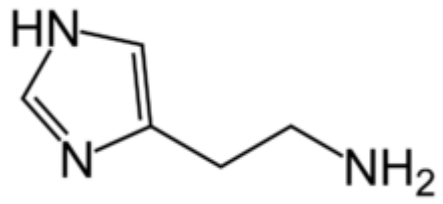
- Charles X, King of France
- James K. Polk, eleventh president of the United States
- Carl von Clausewitz, Prussian soldier and German military theorist
- Elliott Frost, son of American poet Robert Frost

Chapter 18

Scombroid Food Poisoning and Listeria

Scombroid food poisoning

Scombroid food poisoning



Histamine

ICD-10	T61.1
ICD-9	988.0
DiseasesDB	31114
eMedicine	emerg/523

Scombroid food poisoning is a foodborne illness that results from eating spoiled (decayed) fish. It is the second most common type of seafood poisoning, second only to ciguatera. However it is often missed because it resembles an allergic reaction. It is most commonly reported with mackerel, tuna, mahi-mahi, bonito, sardines, anchovies, and related species of fish that were inadequately refrigerated or preserved after being caught. Scombroid syndrome can result from inappropriate handling of fish during storage or processing. One of the toxic agents implicated in scombroid poisoning is histamine. Other chemicals have been found in decaying fish flesh, but their association with scombroid fish poisoning has not been clearly established.

Causes

Unlike many types of food poisoning, this form is not brought about by ingestion of a bacterium or virus. Histidine exists naturally in many types of fish, and at temperatures above 16°C (60°F) on air contact it is converted to the biogenic amine histamine via the enzyme histidine decarboxylase produced by enteric bacteria including *Morganella morganii* (this is one reason why fish should be stored at low temperatures). Histamine is

not destroyed by normal cooking temperatures, so even properly cooked fish can be affected. Histamine is a mediator of allergic reactions, so the symptoms produced are those one would expect to see in severe allergic responses. The suspect toxin is an elevated level of histamine generated by bacterial breakdown of histidine in the muscle protein through elevated production of the enzyme histidine decarboxylase. This natural spoilage process is thought to release additional by-products, which cause the toxic effect. Freezing, cooking, smoking, curing and/or canning do not destroy the potential toxins.

Symptoms

Symptoms consist of skin flushing, throbbing headache, oral burning, abdominal cramps, nausea, diarrhea, palpitations, a sense of unease, and, rarely, collapse or loss of vision. Symptoms usually occur within 10-30 minutes of ingesting the fish and generally are self-limited. Physical signs may include a diffuse blanching erythema, tachycardia, wheezing, and hypotension or hypertension. People with asthma are more vulnerable to respiratory problems such as wheezing or bronchospasms. Symptoms of poisoning can show within just minutes, and up to two hours, following consumption of a spoiled dish. Symptoms usually last for approximately four to six hours and rarely exceed one to two days.

Initial

The first signs of poisoning suggest an allergic reaction with the following symptoms:

- facial flushing/sweating
- burning-peppery taste sensations in the mouth and throat
- dizziness
- nausea
- headache
- tachycardia
- cold like symptoms

Additional symptoms

The above symptoms can advance to the following:

- facial rash
- hives (urticaria). Scombroid hives do not include weals (patchy areas of skin-swelling) that may be seen in true allergies.
- edema (this is generalized if it occurs at all)
- short-term diarrhea
- abdominal cramps.

Severe

In the worst cases, the poisoning may cause:

- blurred vision
- respiratory stress
- swelling of the tongue

Treatment

Treatment is in the form of supportive care such as fluids and oxygen. H1 and H2 receptor (histamine receptors) blocking medications can also be given with some success.

Listeria

Listeria	
Scientific classification	
Kingdom:	Bacteria
Division:	Firmicutes
Class:	Bacilli
Order:	Bacillales
Family:	Listeriaceae
Genus:	*Listeria* Pirie 1940
Species	

L. grayi
L. innocua
L. ivanovii
L. monocytogenes
L. seeligeri
L. murrayi
L. welshimeri

Listeria is a bacterial genus containing six species. Named after the English pioneer of sterile surgery Joseph Lister, the genus was given its current name in 1940. *Listeria* species are Gram-positive bacilli and are typified by *L. monocytogenes*, the causative agent of listeriosis.

Listeria ivanovii is a pathogen of ruminants, and can infect mice in the laboratory, although it is only rarely the cause of human disease.

Listeria monocytogenes

Listeria monocytogenes is a bacterium commonly found in soil, stream water, sewage, plants, and food. Each bacterium is Gram-positive and rod-shaped. *Listeria* are known to be the bacteria responsible for listeriosis, a rare but potentially lethal food-borne infection: The case fatality rate for those with a severe form of infection may approach 25% (*Salmonella*, in comparison, has a mortality rate estimated at less than 1%). Although *Listeria* has low infectivity, it is a hardy bacterium and able to grow in temperatures ranging from 4°C (39°F) (the temperature of a refrigerator), to 37°C (99°F), (the body's internal temperature). Listeriosis is a serious illness, and may manifest itself as a meningitis, or affect the newborn due to its ability to penetrate the endothelial layer of the placenta. Vegetables can become contaminated from the soil, and animals can also be carriers. Listeria has been found in uncooked meats, uncooked vegetables, unpasteurized milk, foods made from unpasteurized milk, and processed foods. Pasteurization and sufficient cooking kill listeria; however, contamination may occur after cooking and before packaging. For example, meat-processing plants producing ready-to-eat foods, such as hot dogs and deli meats, must follow extensive sanitation policies and procedures to prevent listeria contamination.

Pathogenesis

Listeria uses the cellular machinery to move around inside the host cell: It induces directed polymerization of actin by the ActA transmembrane protein, thus pushing the bacterial cell around.

Listeria monocytogenes, for example, encodes virulence genes that are thermoregulated. The expression of virulence factor is optimal at 37 degrees Celsius and is controlled by a transcriptional activator, PrfA, whose expression is thermoregulated by the PrfA thermoregulator UTR element. At low temperatures, the PrfA transcript is not translated due to structural elements near the ribosome binding site. As the bacteria infect the host, the temperature of the host melts the structure and allows translation initiation for the virulent genes.

Listeria monocytogenes is a Gram-positive, rod-shaped bacterium. It is the agent of listeriosis, a serious infection caused by eating food contaminated with the bacteria. The disease affects primarily pregnant women, newborns, and adults with weakened immune systems. Listeriosis is a serious disease for humans; the overt form of the disease has a mortality greater than 25 percent. The two main clinical manifestations are sepsis and meningitis. Meningitis is often complicated by encephalitis, a pathology that is unusual for bacterial infections. Under the microscope, *Listeria* species appear as small, Gram-positive rods, which are sometimes arranged in short chains. In direct smears, they may be coccoid, so they can be mistaken for streptococci. Longer cells may resemble corynebacteria. Flagella are produced at room temperature, but not at 37°C. Hemolytic activity on blood agar has been used as a marker to distinguish *L. monocytogenes* among other *Listeria* species, but it is not an absolutely definitive criterion. Further biochemical

characterization may be necessary to distinguish between the different *Listeria* species. As Gram-positive, nonsporeforming, catalase-positive rods, the genus *Listeria* was classified in the family Corynebacteriaceae through the seventh edition of Bergey's Manual. The 16S rRNA cataloging studies of Stackebrandt, et al. demonstrated that *L. monocytogenes* is a distinct taxon within the Lactobacillus-Bacillus branch of the bacterial phylogeny constructed by Woese. In 2001, the Family Listeriaceae was created within the expanding Order Bacillales, which also includes Staphylococcaceae, Bacillaceae and others. Within this phylogeny, there are six species of *Listeria*. The only other genus in the family is *Brochothrix*.

Mechanism of infection

The majority of *Listeria* bacteria are targeted by the immune system before they are able to cause infection. Those that escape the immune system's initial response, however, spread though intracellular mechanisms and are, therefore, guarded against circulating immune factors (AMI).

To invade, *Listeria* induces macrophage phagocytic uptake by displaying D-galactose in their teichoic acids that are then bound by the macrophage's polysaccharide receptors . Other important adhesins are the internalins. Once phagocytosed, the bacterium is encapsulated by the host cell's acidic phagolysosome organelle. *Listeria*, however, escapes the phagolysosome by lysing the vacuole's entire membrane with secreted hemolysin, now characterized as the exotoxin listeriolysin O. The bacteria then replicate inside the host cell's cytoplasm.

Listeria must then navigate to the cell's periphery to spread the infection to other cells. Outside of the body, *Listeria* has flagellar-driven motility, sometimes described as a "tumbling motility." However, at 37°C, flagella cease to develop and the bacterium instead usurps the host cell's cytoskeleton to move. *Listeria*, inventively, polymerizes an actin tail or "comet" , from actin monomers in the host's cytoplasm with the promotion of virulence factor ActA. The comet forms in a polar manner and aids the bacteria's migration to the host cell's outer membrane. Gelsolin, an actin filament severing protein, localizes at the tail of *Listeria* and accelerates the bacterium's motility. Once at the cell surface, the actin-propelled *Listeria* pushes against the cell's membrane to form protrusions called filopods or "rockets". The protrusions are guided by the cell's leading edge to contact adjacent cells, which then engulf the *Listeria* rocket and the process is repeated, perpetuating the infection. Once phagocytosed, the *Listeria* is never again extracellular: it is an intracytoplasmic parasite like *Shigella flexneri* and *Rickettsia*.

Epidemiology

The Center for Science in the Public Interest has published a list of foods that have sometimes caused outbreaks of *Listeria*: hot dogs, deli meats, raw milk, cheeses (particularly soft-ripened cheeses like feta, Brie, Camembert, blue-veined, or Mexican-

style “queso blanco”), raw and cooked poultry, raw meats, ice cream, raw vegetables, raw and smoked fish and the green lip mussel.

Prevention

The prevention of *Listeria* as a food illness involves effective sanitation of food contact surfaces. Alcohol has proven to be an effective topical sanitizer against *Listeria*. Quaternary ammonium can be used in conjunction with alcohol as a food contact safe sanitizer with increased duration of the sanitizing action. Refrigerated foods in the home should be kept below 4°C (39°F) to discourage bacterial growth. Preventing listeriosis also can be done by carrying out an effective sanitation of food contact surfaces.

Modern relevance/future research

Listeria is an opportunistic pathogen: It is most prevalent in the elderly, pregnant mothers, and AIDS patients. With improved healthcare leading to a growing elderly population and extended life expectancies for AIDS patients, physicians are more likely to encounter this otherwise-rare infection (only 7 per 1,000,000 healthy people are infected with virulent *Listeria* each year). Better understanding the cell biology of *Listeria* infections, including relevant virulence factors, may help us better treat Listeriosis and other intracytoplasmic parasites. Researchers are now investigating the use of *Listeria* as a cancer vaccine, taking advantage of its "ability to induce potent innate and adaptive immunity."

Treatment

Antibiotics effective against *Listeria* species include ampicillin, vancomycin (unclear effectiveness), ciprofloxacin, linezolid, azithromycin.

Intralytix, a Baltimore, Maryland-based biotechnology firm has created a product that combines six different bacteriophages (viruses that infect bacteria exclusively) and is applied to food and areas associated with food production. It has been shown to be effective in the elimination of *Listeria monocytogenes* bacteria.

EBI Food Safety has created and put a similar product on the market, LISTEX P100. LISTEX P100 reduces *Listeria monocytogenes* in food by using bacteriophages to kill them.