
4 Viruses

Viruses are the smallest living things. There is serious scientific discussion about whether they are just a collection of chemicals or whether they are actually living. Many scientists now regard them as life, but in its simplest, most economical form. Usually living organisms have a myriad enzymes, structural proteins, lipids, complex sub-cellular structures and almost unbelievably complex biochemical processes to provide the energy to allow growth, development and reproduction. Viruses short cut all of this by living inside host cells and utilising their biochemical processes to survive and reproduce. The primary purpose of a virus is to infect a cell and to reproduce. A successful virus will not kill its host cell, or at least not until after it has successfully reproduced, and released more viruses to infect more cells and start the process over again.

The Discovery of Viruses

Pasteur's work on bacteria showed that fine filters would retain bacteria, so it was possible to filter out infectivity – this is still used today as a means of sterilising liquids using very fine (e.g. Millipore®) filters. In 1882 Dmitry Ivanowski (1864–1920), a Russian microbiologist, studied tobacco mosaic disease and showed that the agent that caused the disease could not be filtered out. Indeed, he showed that cell-free filtrates could cause the disease in healthy tobacco plants. When he tried to culture this disease

causing principle in culture media used for bacteria, he found that no growth occurred. So he had isolated a very small (i.e. it would pass through a filter that retained bacteria and other cells) disease causing agent that could not be cultured. A prominent Dutch scientist, Martinus Beijerinck (1851–1931), confirmed these findings and called the infectious filtrate “living infectious fluid”. Pasteur was investigating rabies at about the same time (1892) and called the filterable agent that caused rabies “virus”. It was about 25 years before instrumentation became sufficiently advanced to allow proper study of the miniscule disease causing “viruses” that Pasteur, Ivanowski and Beijerinck had described. By this time it was known that viruses could not be grown outside living cells. It is for this reason that most of the work on the structure and infectivity of viruses was done on bacteriophages because they were easy to culture in bacteria. At this point in scientific history it was not possible to culture mammalian cells and therefore human and animal viruses could not be cultured outside living creatures.

It was not until the 1960s when the electron microscope was introduced, and animal cell culture techniques became available that the first images of viruses were seen. They were a marvel; beautiful, symmetrical, structures containing the rudimentary principles of life. But were they alive, or were they just a collection of chemicals?

Some of the Worst Diseases are Caused by Viruses

Viruses can be very nasty indeed. They cause some of our most feared diseases. Smallpox, Ebola, Acquired Immune Deficiency Syndrome (AIDS), Severe Acute Respiratory Syndrome (SARS), and polio are all viral diseases. Some cancers are caused by viruses – I’ll talk about these later. Worst still, no viral disease is curable by medicines, although there are now pharmaceuticals available that slow down, or inhibit, viral reproduction so slowing down the course of the disease that the particular virus causes. An example of such a medicine is Acyclovir used in the treatment of AIDS and some hepatitis virus infections. Usually,

as with the common cold, you just have to wait until the body's own defences (the immune system) overpowers the virus. Contrary to popular belief, antibiotics have no effect on viruses. Viruses are the ultimate infectious agents, they can't be treated, and some trick our immune system into not recognising them, or interfere with the process of immunity in order to allow themselves to reproduce without being wiped out by antibodies produced by their host. AIDS works in this way. It suppresses the immune system which is why it is so devastating.

The picture of viruses that I have painted so far is particularly grim, but there are some good viruses. These are a group of very specifically adapted viruses that attack and kill bacteria. They are called bacteriophages, or phage for short. They are becoming increasingly important as a potential means of destroying pathogenic bacteria, for example on food. I'll return to this later too.

What is a Virus? How Does it Infect a Cell?

There are several types of virus, but basically they all consist of a lipoprotein coat with nucleic acid inside. They are classified according to the type of nucleic acid that they contain. So there are DNA (deoxyribosenucleic acid) viruses and RNA (ribosenucleic acid) viruses. They are incredibly small; most are between 20 and 400 nm (a nanometre is 0.00000001; 1/1,000,000,000; 10^{-9} , or a thousand millionth of a metre). This is the size of a large molecule, and therefore very powerful electron microscopes are needed to see them (Fig. 4-1).

To infect a cell the virus lipoprotein coat fuses with the cell membrane (a protein lipid "wall" that holds the cell together) releasing its nucleic acid (DNA or RNA) into the cell. The nucleic acid then incorporates into the cell's own genome (i.e. collection of genes also made from DNA) and gets replicated when the cell switches on its own DNA replication. The viral nucleic acid codes for its own lipoprotein coat, so that as the host cell replicates DNA and makes proteins from the DNA template it produces all that the virus needs to make more viruses. When

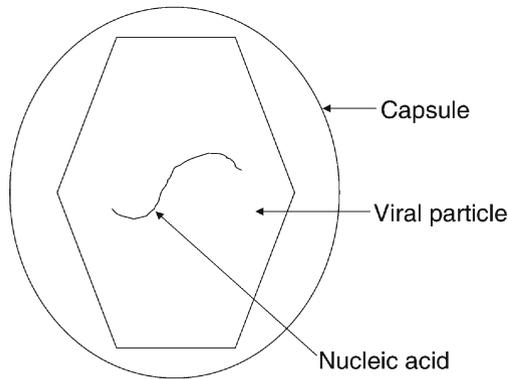


Fig. 4-1. The structure of a typical virus

the virus has replicated the host cell holds many thousands of viral offspring. At this point the cell dies, ruptures and releases the viruses to infect surrounding cells, or be expelled from the body and infect a new unsuspecting host. This is what happens when you have a cold, you sneeze out viruses that are breathed in by someone else and grow in the new host's cells.

Viruses and Food

Viruses are gaining increasing importance as food borne vectors of disease. It is estimated that in the USA at least 75% of food poisonings are caused by viruses. Many viruses cause rapid onset, short, sharp illness, and so by the time a sufferer gets round to going to their doctor the symptoms have subsided. For this reason the vast majority of food borne viral disease is not reported, and therefore it is not possible to estimate its real incidence.

There are several viruses important in food safety. I'll look at them individually.

Noro Virus

This used to be called Norwalk-like virus – it was re-named in 2002; Norwalk is a town in Ohio, USA where the first outbreak occurred in 1968. It is a member of a family of viruses called the *Caliciviruses* which have their genetic material on single stranded DNA.

As we learn more about the virus it is becoming clear that it is probably the most prevalent food poisoning organism world-wide. In the USA there are at least 180,000 confirmed cases per year, and it is estimated that 60% of the US population is exposed to the virus by the age of 50. It has been estimated that in the USA there are 9,200,000 Noro virus cases per year (i.e. 3,150/100,000 population/yr, or an incidence of 3% disease in the population). This makes Noro virus by far the commonest form of food poisoning.

There are a range of Noro viruses, each is slightly different (different serotypes, i.e. they cause different antibodies to be formed following inoculation of animals/humans), but they all cause pretty well the same symptoms. Typical symptoms of Noro infection are vomiting and watery diarrhoea, sometimes with stomach cramps and nausea, and less frequently with fever. The virus acts quickly – symptoms begin 24–48 hours after infection, and have usually cleared up within a further 24 hours. Death has been reported in rare cases where the sufferer was unable to replace the fluids lost quickly enough.

Noro viruses can only live and reproduce in human cells. The virus is present in the faeces of infected people, therefore the faecal/oral route is the most important transmission route. This is the typical food borne illness transmission route – an infected person goes to the toilet, does not wash their hands properly (if at all) then handles food. The consumer of the food is very likely indeed to get infected. The worst scenario is a person who is infected, but has not started to show the symptoms and therefore does not know that they present a particular hazard if they work in the food industry.

There are numerous examples of Noro virus outbreaks. Most are in institutions, cruise ships, or in hotels. There are “captive audiences” here, all ready and waiting to be infected! All

that it takes is one infected person to handle food and the virus will spread through the “closed” community very quickly indeed. Another faecal/oral infection route is via shellfish that might be growing in infected waters.

Shellfish

New Zealand had a particular problem with this route in 2001/02 when mussels and oysters grown in Northland were contaminated because pleasure boats moored near to the shellfish farms were discharging untreated sewage, Noro virus in the sewage contaminated the sheltered harbour, and the virus was filtered by the shellfish. Anyone eating the uncooked shellfish was at significant risk of infection. The contamination rate was so high that the shellfish farms were closed until the problem could be solved. They were still closed in 2004.

A specific case illustrates the problem well. Thirty-six of 95 people (38%) attending a yacht club Christmas celebration in the north of North Island, New Zealand in December 1994 went down with gastrointestinal illness likely to be Noro virus. The oysters were very likely to have originated from the Bay of Islands in Northland, New Zealand. There are numerous similar epidemiological examples that point to oysters and the Bay of Islands, hence the draconian, but justified, action to close the fisheries (see also page 91).

Cruise Ships

An outbreak of Noro virus infection occurred on the Star Princess which left Seattle in August 2003. Within the first 2 days of the cruise 60 of the ship’s 2,800 passengers (2%) reported gastrointestinal upset. Clearly something was wrong! Simple hygiene regulations were activated which resulted in prevention of further spread of the virus. The source of the infection is not known, but it is most likely that one of the kitchen staff was infected, or less likely that the infection originated in a passenger who handled food that others ate.

Restaurants

There have been numerous cases of Noro virus infection originating from an infected staff member in fast food outlets. Less commonly diners are implicated, but there is one very good example of this, it occurred in Derby, UK in a hotel dining room. A diner was suffering from Noro virus infection and vomited in the restaurant. Within 48 hours over 60% of the restaurant's diners were suffering from gastroenteritis. The most likely transmission route here is virus-containing droplets originating from the vomit landing on the diners meals. This is supported by the fact that the diners nearer to the woman were more likely to get ill; 90% of the people at the same table were infected.

Retirement Homes

This is another captive population, and there are numerous cases of Noro virus infection sweeping through rest and retirement homes. The problem here is that the residents are likely to be severely affected; they are old, less likely to produce a good immune response, and more likely to suffer the ill-effects of dehydration. These are the occasions that are most frequently associated with fatalities.

How to Prevent Infection

There are a set of simple rules (formulated by the Virginia Department of Health, USA) that food handlers, and others should follow to reduce the risk of the spread of Noro virus infection:

- Wash your hands frequently
- Promptly disinfect contaminated surfaces with household chlorine bleach based cleaners
- Wash soiled clothing
- Cook oysters completely to kill the virus
- Avoid food (or water) from sources that may be contaminated.

Hepatitis Viruses

Hepatitis (from the Greek, *hepatikos* – liver) is inflammation of the liver. It can be caused by chemicals (e.g. alcohol), bacteria or viruses. Only viral hepatitis will be discussed here.

There are 5 different hepatitis viruses, they are called Hepatitis A, B, C, D and E. Only HepA can be food borne, but this is not its only means of transmission.

HepA

The HepA virus (HAV) is a member of the family of viruses called the Picornaviridae, they have a single RNA molecule surrounded by protein capsule (called a capsid) with a diameter of 27 nm. Most of the Picornaviridae cause disease, others include the polioviruses and rhinoviruses that cause the common cold.

HepA is not a serious disease – unlike the other types of hepatitis which are often very serious, and might have long term effects like cancer or cirrhosis. Some might be fatal. The symptoms of HepA include sudden onset of fever, malaise, nausea, anorexia, and abdominal discomfort, followed after a few days by jaundice (yellowing of the skin and whites of the eyes). The disease usually lasts for less than 2-weeks with complete recovery. Only 10–100 viral particles are needed to cause infection.

HAV lives and reproduces in the liver and is secreted into the bile which is released into the intestine via the bile duct. The virus can survive in the intestine and passes through with the food, eventually being expelled in the faeces. For this reason faeces from HepA sufferers are infectious.

Route of Infection

HepA is primarily a food borne disease. A HepA sufferer who is not hygienic after going to the toilet and then handles food is likely to leave viruses behind on the food. This is the same sto-

ry as for Noro virus. Don't forget that it takes less than 100 virus particles to cause an infection. Faeces from an infected person would harbour many millions of viruses.

HAV appears to be more stable than many other viruses. Most viruses don't survive long outside a host cell, however HAV can survive for days, so contaminated food remains infectious for a few days, if not longer. Cooking kills the virus, so transmission usually involves foods that are not cooked (e.g. fruit picked by infected people), or cold cuts of meat.

HepA in Blueberries

New Zealand's North Island is a producer of excellent blueberries. At Christmas 2001 there were several cases of HepA in New Zealanders. This was not considered to be of any great importance initially because we would expect a few cases of HepA in the population each year, and the disease is not serious. However as the number of cases rose to 29, the Health Authorities became more interested because of the possibility of a point source of contamination, and the need to eliminate the source in order to prevent further spread of the infection.

A great deal of good epidemiological work led to identification of a common factor amongst infected people – blueberries. In fact 17 of the 29 cases (59%) were shown to be associated with consumption of blueberries. Six blueberry samples were examined in the laboratory and were found to be contaminated with HAV. It turned out that a blueberry grower had a child who had HepA, in addition the grower had no toilet facilities near to the berry fields and so the pickers relieved themselves in the field. Human faeces were found in the field too, the rest is a bit unclear, but either the child infected the blueberries, or infected pickers, or both. The outcome was serious and required urgent attention. We had a situation in which a HepA-infected person might poo in the field, could not wash their hands, then might pick blueberries, and transfer virus from the faeces to the blueberries via their contaminated hands. In addition, a nice steaming reservoir of the virus was left at the edge of the field! (Fig. 4-2).



Fig. 4-2. Picking blueberries (*photo by Margaret Tanner*)

When blueberries are eaten raw a lot of people don't wash them because they like to see the lovely grey bloom on the fruit. In this scenario this would have increased the risk of infection. We don't know whether washing would remove all of the virus particles, but it is likely to remove some and so decrease the risk of infection from eating the contaminated fruit.

HepA in Raspberries

Fruits are a good vector for HepA and there have been several cases of their involvement in transmission of the disease. Another example was in Scotland ten or so years ago when contaminated raspberries were fed to a medical conference as a seasonal treat from a part of the world renowned for its raspberries. A significant number of the conference delegates contracted HepA ... rough justice!

Other Viruses

There are other viruses that might be transmitted to people via food but we do not regard them as conventional food borne viruses (partly because their transmission is rare, and partly because food is not their primary route of transfer). Shellfish are good vectors for many viruses because they filter their food from sea water, therefore if there is a virus (or a bacterium, or other pathogen) in the water the shellfish might filter it out along with its food. The unsuspecting consumer of the raw shellfish might contact the viral disease.

The polio virus is a good example of a water-borne virus that might contaminate sea water. But where does it come from? The most likely source is faeces dumped from boats and ships. Fortunately polio is relatively rare in the developed world and therefore it is not very likely that the virus will be present in the vicinity of shellfish fisheries. Despite this we do have worries about the possibility, especially as the polio virus can withstand some of the preservation methods for shellfish (e.g. marinating mussel). Cooking will kill most viruses, so these concerns apply only to uncooked shellfish.

A possible scenario of infection illustrates this potential problem well. In the north of New Zealand's North Island there is an idyllic place called Bay of Islands. This boatie's wonderland attracts many tourists who spend their time sailing around the bay, eating nice food, and drinking wonderful New Zealand wines. Heaven! The Bay of Islands is also an excellent shellfishery. Both feral molluscs and shellfisheries are important sources of green lipped mussels and oysters. The problem is that some of the boaties discard their untreated waste overboard – viruses and all. This is a problem from the point of view of conventional food-borne viruses such as Noro virus and HAV, but it could also be a route for other viruses to enter the food chain if one of the boaties harboured, for example, polio virus. The New Zealand authorities were sufficiently worried about the problem to close the Bay of Island fisheries and make it illegal to take shellfish for human consumption (Fig. 4-3) (see also page 86).

There are many other viruses that could be transmitted in this way, most of which we haven't even thought about.

NEW ZEALAND



Fig. 4-3. New Zealand showing Bay of Islands

Viruses Are Not All Bad

As I mentioned at the beginning of this Chapter, there is a very interesting family of viruses called bacteriophages or phages for short. They are the goodies of the viral world because they infect and kill bacteria. Phages are specific to a bacterial species, e.g. T4 Phage infects only *E. coli* (Fig. 4-4 and Fig. 4-5).

Phages are spectacular viruses, they are built like microscopic Lunar Landing Modules. They dock onto the surface of

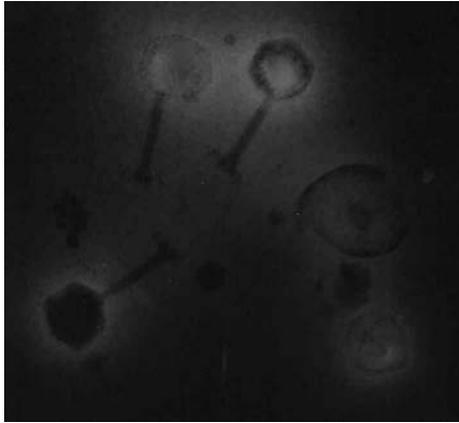


Fig. 4-4. Electron micrograph of bacteriophages. These amazing “creatures” infect cells by injecting their nucleic acid into the host cell. The phage on the left has a white head because it has injected its nucleic acid into its host electron micrograph taken by Manfred Ingerfeld at the University of Canterbury, New Zealand and kindly provided by Gwyneth Carey-Smith

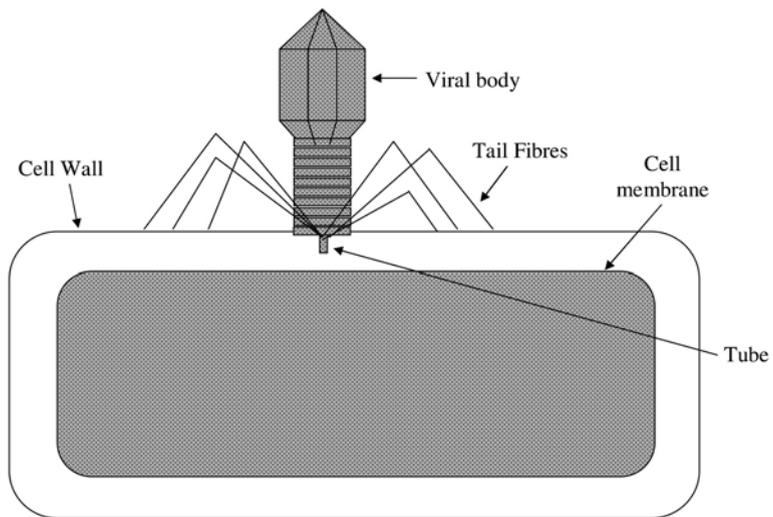


Fig. 4-5. A bacteriophage docked on a bacterial cell surface

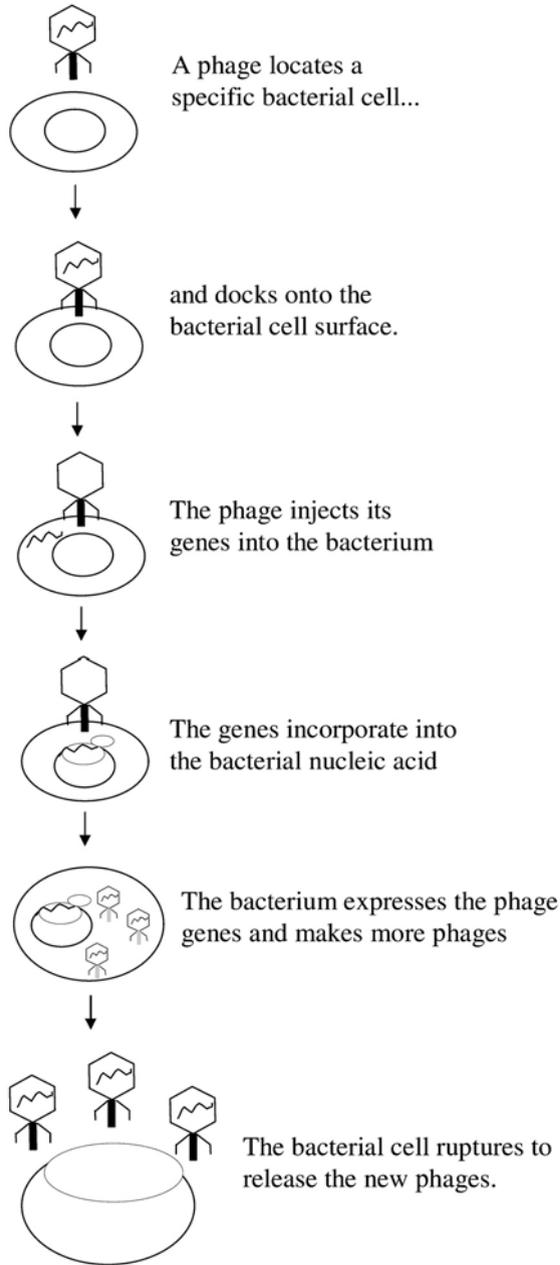


Fig. 4-6. The process of phage infection

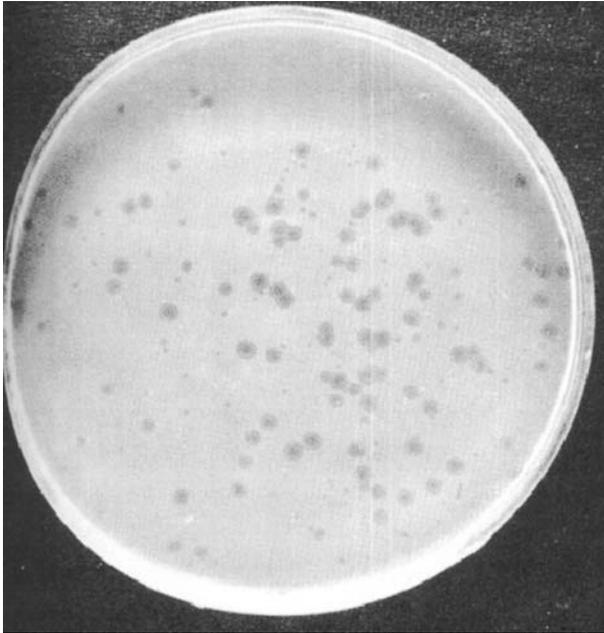


Fig. 4-7. Phage plaques on an agar plate (*photograph kindly provided by Gwyneth Care-Smith, ESR, New Zealand*)

the bacterium-using specific bacterial cell surface proteins to identify their host. Then inject their genetic material into the bacterium, the bug then replicates the virus and breaks open (lyses) to release millions of new phages that infect the surrounding bacterial cells (Fig. 4-6).

It is easy to demonstrate this effect in the lab by infecting a bacterial culture with phage. The phages infect the bacteria growing on the Agar plate and kill them creating zones of dead bacteria called plaques. You can see these in the picture (Fig. 4-7).

Scientists are just beginning to see the value of these viruses as a means of making our food safe. One of the most problematic food-borne pathogens is *Campylobacter*. If we could find a Campy phage we could infect food with the phage and let the phage kill any Campy on the food and so make the food safe. This is a brilliant idea. In a few years I think that ex-

actly this will be done. Looking even further ahead, it might be possible to infect farm animals, or entire farms, with pathogen-specific phages so wiping out food-pathogens on the farm. This might be a pipe dream, but it is a wonderful idea ... watch this space.

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Mad Cow Disease and the Elusive Prion