VIRUSES, KOALAS AND LEUKAEMIA

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Are viruses alive? The jury is still out – however regardless of their taxonomic status no other entity has had more effect on the evolution and ecology of life on the planet. Every species - plant, animal or bacteria - examined so far has its own associated viruses which have diverse impacts both on individual hosts and host populations. The devastation of New World indigenous peoples by smallpox between 1600 and 1900 and the more recent emergence of HIV-AIDS are two graphic reminders of how viruses shape populations.

Viruses were first discovered by two Danish investigators in 1908. Ellerman was a physician and Bang a veterinarian and together they were attempting to track down the cause of leukaemia in chickens. They soon realized that whatever was causing the disease in chickens was much smaller than any known microbe and hence the study of viruses was born. It was another 50 years before a virus was seen with the electron microscope and then scientists began to understand just how different viruses were from other forms of life. In the mean time Virology stumbled along as an esoteric offshoot of pathology and this fostered beginning unfortunately left a narrow bias on the field. Viruses became equated with disease. What they did when not causing disease and how they fitted into the planets ecology remained a black box. Only in the last 15 years have investigators started to look more broadly at virus ecology and are now realizing that while viruses sometimes cause disease they also underpin the diversity of life in fundamental ways.

What are Viruses

Viruses are small. They range in size from 100-1000 nanometres (millionths of a millimetre) and vary in shape from spherical to long filaments. They consist of small lengths of genetic material (DNA or RNA) packed with protective proteins and surrounded by a protective membrane. Their genetic material consists of a few genes which contain the information or blueprint necessary to make more viruses. The surrounding membrane, called the viral envelope, has two main functions. It protects the genetic material while the virus is floating around in the environment and it directs the virus to the correct host. Unlike other living cells or organisms viruses can't reproduce by themselves. They contain only the blueprint genes necessary to direct their reproduction but in keeping with their small size they lack the necessary machinery and raw materials to actually construct themselves. So in order to reproduce, viruses need the help of a host cell. The host maybe an animal, plant or bacteria. The virus enters the correct host species and cell type via molecular "key-like" structures that project out from the envelope. These structures find and match corresponding "molecular locks" called receptors that are present on the surface of the host cell. The viral keys only match a specific range of receptors. So this function determines the host species and tissue type that can be infected by a particular virus. After the virus "locks" onto the correct cell type it enters the cell and switches on the viral genetic program. The virus overrides the cells genetic program and subverts the cellular machinery into making multiple copies of new virus. These are excreted back out into the environment where they can infect the next suitable host. Not all viral progeny are exact copies of the parent virus when they are released from the cell. In fact many virus produce "swarms" of progeny that have slight differences in their structure and function. This is particularly important with respect to the "key" molecules on the surface of the viral envelope; by producing swarms with a range of slight changes in the key structures the virus can potentially extend its host range. Under some circumstances viruses can pick up pieces of host DNA during replication which are then carried by the viral progeny. In this way viruses can act as vehicles to exchange "genes" between individuals and even between species. Technology has capitalized on this process; we can now construct synthetic viruses containing genes of interest and use them to deliver these genes into plant or animal tissues for therapeutic or agricultural purposes.

Outcomes of Viral infection

There are several possible outcomes to the process of viral replication within the host cell. If the virus is particularly aggressive in its take over of the cell it may destroy the cell completely. Clearly if this happens quickly and on a large enough scale the result would be major tissue/organ damage and possible death of the host. Ebola virus infection is a good example of this type of infection. On the other hand HIV seems to tick away in the background replicating itself in the host for a long period of time before the AIDS syndrome and obvious disease occurs. At the other end of the scale viruses such as the Spumaviruses are particularly well adapted to their hosts and replicate effectively without causing any detectable ill health over the life time of the host. The latter seems to be the best strategy as it ensures the virus has as safe place to live and reproduce for a long time. Some of the retroviruses have taken this strategy to the extreme. Not only do they enter the host cell but they insert a copy of themselves into the host DNA. When this occurs in host germ line tissue the virus then becomes part of the host genetic material and is passed on to all subsequent host generations just like a normal host gene. This is called endogenisation and represents the ultimate in genetic symbiosis. Recently the complete human genome (total genetic material) was sequenced and surprisingly about 10% of it is derived from viral DNA. Some of this material is also present in the genomes of apes indicating that these viruses have been hitching a ride on our DNA for millions of years!

Viral Symbiosis

Some host virus-relationships have evolved even further into the arena of mutual benefit. Not only is the virus harmless to its host but its presence and function confer some benefit. Early in the evolution of higher primates a virus entered the germ line and "switched on" the amylase gene in salivary gland tissue. Amylase is an enzyme used to break down starch. It is theorized that this new found ability to produce amylase in saliva gave higher primates the capacity to chew and break down starchy cereal grains more effectively. This in turn contributed to the early hominid apes being able to move out of arboreal habitats into grassland systems. On a similar theme, the recently discovered human gene, "Syncytin", was found to be critical in the proper development of the placenta during pregnancy. When investigators looked closely at the gene sequence they found, much to their surprise, that it was a viral envelope gene. Some time in the past a virus invaded the germ line and while most of the virus was subsequently lost the host decided to co-opt the viral envelope gene and use it to help build placentas for developing embryos. These examples of how virus have changed gene expression and contributed new genes to a species highlight their role in causing behavioral and physiological change in a species over evolutionary time. In short by transferring genes in this way they speed up evolution.

On a broader scale some animal populations use viruses as a way of protecting their range and distribution. This phenomenon has been termed "aggressive symbiosis" and is particularly evident in the relationship between Samairi monkeys and their herpesvirus and also between old world primates and their Simian Immunodefeciency Viruses (SIV). In each case a significant number of the host population is infected by the virus which is actively replicating but in that host never causes disease. If however their territory is infringed on by a related competitor species the virus is transferred (usually by close contact) to the invading animals where it causes severe and sometimes rapidly fatal disease. In these cases host and virus have co-evolved to a point where they have worked out that co-operation is better that competition. The virus has a nice safe refuge in which to replicate for as long as the population survives; and the host has a biological weapon with which to protect its niche.

However the most startling example of virus host symbiosis occurs in insects. Braconid and Ichneumonid wasps comprise about 100000 species and have been co-evolving with Polydnaviruses for about 75 million years. The viruses are integrated into their germ line and are passed from generation to generation like a normal host gene set. The viruses only become activated in the ovarian tissue of the female wasp at the time she lays eggs. These wasps lay their eggs into the larval stages of other insects and the wasp eggs rely on their insect larval prey for security and nourishment during development. While the female lays her eggs into the prey species the activated polydnaviruses in her ovaries replicate producing multiple completely packaged viruses. These are then co-injected into the prey species with the wasp's eggs where they set about producing a variety of chemicals that compromise the preys immune system. Thus the virus ensures the safety of the eggs until they hatch.

Environmental Viruses

Over the past 15 years virologists have moved out into the environment to try an estimate the range and abundance of viruses and how they fit into general ecology. Most of this research has been carried out in marine and freshwater environments and has yielded some bizarre results. On average there are 10 million viruses per ml of water in the general environment. Most of these are associated with the huge populations of bacteria, algae and other single celled organisms that inhabit marine and freshwater systems. By infecting and lysing selected portions of these populations viruses act as pruning and containing agents and in the process release and recycle a vast amount of nutrient on a daily basis. A closer examination of the genetic sequence of the viruses shows that 70% of the genes that they carry have no known counterparts in the animal and plant genetic databases. This implies that environmental viruses are an archive of genetic material that sits at the base of the food chain. Interestingly some genes found in viruses from marine systems off the west coast of America were identical to those found from freshwater streams in Europe. This suggests that viral gene transfer between hosts is occurring on a global scale. These observations have lead scientists to consider that viruses form a global community responsible for the transfer of genetic material within and between species. This "Viriosphere" is like a large web and communications network that is the genetic base for life on earth. Because the viriosphere provides a huge genetic reservoir it means that host populations have access to a greater range of genetic tricks to help them cope with a changing environment. It also means that evolution and adaptation are not restricted to slow gradual changes over time but can progress in rapid bounds.

Summary so far

Viruses are genetic elements that must live and replicate in association with a host. They are ubiquitous in the environment and provide a large gene pool and gene transfer network for all living things. This in turn contributes to evolutionary change and increases genetic variability within populations enhancing adaptability. In aquatic environments viruses control populations of microscopic life and contribute to nutrient recyclying. The virus host relationship can take many forms. Viral infection is often benign to the host as survival of the host is in the best interest of the virus. Sometimes the relationship is mutually beneficial. This is most common in situations where virus and host have co-evolved over long periods. Sometimes viruses cause disease in the host. This is more common in situations where virus and host are new to each other.

Koala Retrovirus and Leukaemia

Field biologists early in the 20th century first noted that koalas seemed to have a high prevalence of leukaemia and lymphoma and many workers since have experienced the fact that some koalas are refractory to treatment and often succumb to infectious disease. In koalas this syndrome has been termed LLIS (Leukaemia, lymphoma and Immune Suppression) This pattern of disease is seen in a number species and is often associated with retroviral infection. The first viruses discovered in leukaemic chickens back in 1908 turned out to be retroviruses. Thus it was no surprise that in 1998 Jon Hanger at the University of Queensland isolated a retrovirus from koalas. The virus however turned out to have some very unusual properties. Firstly although not all koalas get this disease almost every koala was infected with the virus. Secondly the virus is integrated into the koala germ line and is being transmitted from parent to offspring like a normal gene. Despite this, the virus appears to be active in every animal. Blood samples from any animal will show free viral particles in varying amounts. Finally the koala retrovirus (KORV) turned out to be almost identical to a leukaemia causing virus found in gibbons. This was totally unexpected and it implies that there has been recent (probably in the last 200 years) cross transmission of virus between koalas and gibbons. More recent studies by Rachael Tarlinton at the University of Queensland have demonstrated that northern koalas approach 100% prevalence with KORV while Victorian koalas vary between 40 and 60%. She has also shown that the level of virus found in the blood of koalas correlates strongly with the severity of disease seen.

These findings have lead to the following hypothesis;

1. The LLIS syndrome is probably due to infection with KoRV
2. KoRV was recently introduced to koala populations. How this has happened remains unclear but is subject to further investigation.
3. KoRV is in the process of becoming endogenised in koalas. That is, KORV is becoming part of koala DNA and has the potential to profoundly affect the species.

The LLIS Syndrome

Neoplasia
Lymphoid neoplasia
Myeloid leukaemia
Osteochondroma and other tumours
Aplastic anaemia and myelodysplasia/preleukaemia
Immune dysfunction

Clinical signs of LLIS in koalas

Animals usually present with some or all of the following:

Enlargement of one or more peripheral lymph nodes or thymus;
Chronic illthrift/poor doers, weight loss;
Excessive drinking
Soil pica;
Diarrhoea;
Paralysis or CNS signs, opportunistic infection; loss of pouch young.
Intermittent drooling and mouth infection
Widespread or chronic infections that wont respond to treatment.

Mortality and the Incidence of Leukaemia and Lymphoma (LL) in various populations. Numbers are cases per 100 animals per year

POPULATION	Mortality	LL	% of mortality
FREE RANGING	22	3.2	15
CAPTIVE	9	6.4	71

Treatment of LLIS in Koalas

There is no specific therapy available to treat the virus. General supportive therapy and care is important to maintain the animals quality of life. Most lymphoid neoplasms in koalas have a rapidly progressive clinical course, requiring euthanasia usually within a few weeks of diagnosis at the most. Surgical removal of a solitary tumour of a superficial cervical lymph node or thymus resulted in clinical remission in one aged koala, who was euthanised two years later with a cranial osteochondroma. In two out of two cases on which chemotherapy was tried, the koalas required euthanasia within one week. High dose prednisolone may cause temporary reduction in clinical signs. Most koalas are given milk formula supplements in the form of a paste, with or without additional vitamins. Antimicrobial and supportive therapies may provide relief for secondary conditions such as stomatitis or dermatitis, but generally it can be expected that aplastic anaemia or myelodysplasia will cause death over a variable period of time. Euthanasia should be considered in cases with intractable infections or when quality of life issues are prominent.

Implications for koala populations and conservation

In a natural context virus commonly cross species sometimes causing epidemics within a population so the recent emergence of KORV and associated LLIS in koala populations by itself is not alarming. However in combination with habitat loss and reduced fertility associated with chlamydiosis, KORV-LLIS may contribute to local extinctions. The fact that virus appears to have only come into koalas in the last 200 years raises concerns that we may have introduced it sometime during the early colonization of Australia.

The table above indicates, from the limited information we have collected so far, that leukaemia and lymphoma are only a minor component of mortality in free ranging populations. Immune suppression however is much harder to assess in wild populations. One interesting observation is that in areas where KORV is less prevalent clinical chlamydiosis is also less prevalent. It is possible that KORV infection makes koalas more susceptible to Chlamydia infection. If this is this case it could have a profound effect on population viability in the short to medium term.

In the long term we would expect that the virus and koalas will reach a truce resulting in a reduction LLIS. Who knows, koalas may eventually inactivate the virus in their DNA or find a beneficial use for it. In captive institutions the situation is more graphic with LLIS representing the major cause of koala mortality. Selective breeding against koalas with high virus levels and or the future development of a vaccine may help address this issue.

Considerations for Wildlife Carers and Professionals

The recent cross-transmission of a leukaemia causing virus between koalas and a primate raises concerns about carer health. Will it jump species again? Could it jump into people? It is certainly possible but there is no effective way of determining where or when. Due care to hygiene and safety while handling animals is paramount. Of more concern is the general process of wildlife rescue and rehabilitation. Viruses are everywhere and are constantly altering their structure in a search for new hosts. The caring process often brings animals into close direct or indirect contact with other individuals or other species – a process that would not naturally occur in the wild. This means that the wildlife care system could act as a focus for the transfer of disease back into wild populations. In the worst case it could accelerate the transfer of viruses between species and result in epidemics.

Several simple precautions can help reduce this possibility and the possibility of carers themselves contracting a nasty disease;

- Always wash thoroughly with antiseptic soap before and after handling an animal
- When handling animals wear protective clothing and disposable gloves and change them before handling another animal. This creates a protective barrier between you and the animal and reduces the chance that you or your clothing will transfer disease agents between animals.
- If animals are solitary in the wild then house them separately while in care. In particular do not house different species together.
- Ensure that chronically ill animals are not released back into the wild.

First, do no harm.

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WEB SITES

Australian Wildlife Health Network, Facts Sheets on Koala Retrovirus
http://www.wildlifehealth.org.au/AWHN/FactSheets/Fact_Display.aspx

Reference and links on viruses and virology in general
http://www.virology.net/