-VE STRAND RNA VIRUSES

References:
Flint. Principles of Virology, Pp 45-46, 178-179, 351-352, 538-540, 756-759
Fields Virology, 4th Edition, Chapters 38 thru 45
http://www.who.int/csr/don/ (WHO Disease Outbreak News)
http://www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/ebola.htm (CDC - Ebola)
http://www.unicef.org/measles/index.html (UNICEF - Measles)
http://www.measlesinitiative.org/index3.asp (Measles Initiative)

The **Mononegavirales** (*mono* - single; *nega* - negative; *virales* - viruses) are a taxomonic order, which includes several families of viruses with similar genomic organization and replicate strategies -- the *Filoviridae*, *Paramyxoviridae* and *Rhabdoviridae*, plus Borna disease virus. These viruses probably diverged from a single common ancestor as recently as the last ice age. They are also frequently associated with **emerging infections** and/or cross-species transmission events (eg, Ebola).

Use of negative sense (-) RNA genomes means, by definition, that the viral genome is of opposite polarity to mRNA. Thus, the viral genome cannot be used to make proteins until it has first been transcribed to produce mRNAs. This has the following implications:
(I) purified virion RNA is not infectious (as noted above, it cannot encode protein)
(II) the viruses must bring their own RNA polymerase into the cell in order to make mRNA (ie, the viral polymerase must be incorporated into the viral particle, or virion)

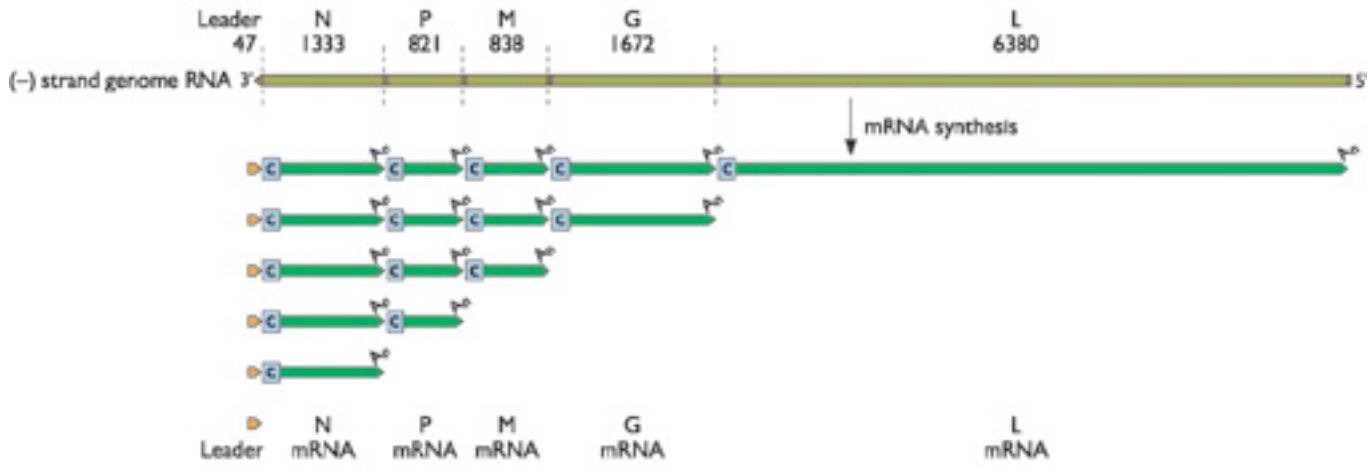
The other key feature of these viruses is that they make **gene-unit length mRNAs** (ie, each mRNA encodes only a single protein). This is achieved by the use of transcriptional stop and start signals, which are located at the boundaries of all of the viral genes. **Stop/start transcription** has two major results:

1). Since there is only a single promoter, located at the 3' end of the viral genome, the polymerase can only load onto its RNA template at one site. As it moves along the viral RNA, the polymerase encounters stop/start signals at the boundaries of each of the viral genes. This results in pausing of the enzyme, which often falls off the template. The result is that more mRNA is made from genes that are located close to the promoter, and less mRNA is made from genes located far from the promoter. This means that there is a **polarity of transcription** (see Figure below). The viruses use this to regulate the expression of their genes, since highly expressed proteins are encoded close to the promoter (eg, structural proteins such as the nucleocapsid protein, N), while proteins that are needed in only small amounts (eg, enzymes such as the RNA polymerase, L) are encoded far away from the promoter.

2). The other major consequence of stop/start transcription is that it complicates genome replication. The only way that the complete viral RNA genome can be copied is if the transcriptional stop/start signals can be ignored or over-ridden. This means that the critical decision during viral RNA synthesis occurs very early on -- at the first gene boundary (located between the leader RNA and the N gene). If the stop/start signals here are obeyed, then only subgenomic mRNAs will be produced. However, if the stop/start signal here is ignored or over-ridden, then a complete copy of the viral genome can be made.

Transcriptional polarity

(Flint. Fig 6.11A)



Mononegavirales

mono - single; *nega* - negative; *virales* - viruses

Included within this order are: *Bornaviridae, Filoviridae*, *Paramyxoviridae* and *Rhabdoviridae*.

Common Features:

1) Genome: linear monopartite (-) RNA
2) Genome organization: 3'-[untrans. leader]-[CORE]-[ENVELOPE]-[POL]-[untranslated]-5'
3) Virion: helical nucleocapsid containing a viral RNA-dependent RNA-polymerase
   - competent for transcription on entry; protein synthesis required for replication
4) Transcription: make 6-10 discrete RNAs by stop/start synthesis from one promoter
   - leader transcripts are different from others: no polyA, no cap
   - transcriptional signals delineate genes: initiate at 3', terminate at 5' (plus polyA)
5) Replication: make a full-length (+) RNA that acts as a template for progeny genomes
   - decision to replicate made at leader/core boundary (read-thru)

Divergent Features

Family	Genome	Morphology	Hosts	Disease
Filo-	7 proteins; 19 kb	Filamentous	Reservoir = ?; *can infect primates*	Hemorrhagic fevers

- Genus: Marburg
- Genus: Ebola - 4 subtypes: EBO-Z [Zaire], EBO-CI [Cote d'Ivoire], EBO-R [Reston], EBO-S [Sudan]

Paramyxo-	10-12 proteins; 15-18 kb	Pleomorphic	Vertebrates	Mainly respiratory

Subfamily: Paramyxovirinae

- Genus: Morbillivirus (e.g., measles virus, canine distemper)
- Genus: Respirovirus (e.g., parainfluenzaviruses [Sendai = PIV-1])
- Genus: Rubulavirus (e.g., mumps virus)
- *Possible new future genus: Henipavirus (e.g., Hendra virus, Nipah virus); largest of paramyxoviruses*

Subfamily: Pneumovirinae

- Genus: Pneumovirus (e.g., respiratory syncytial virus)

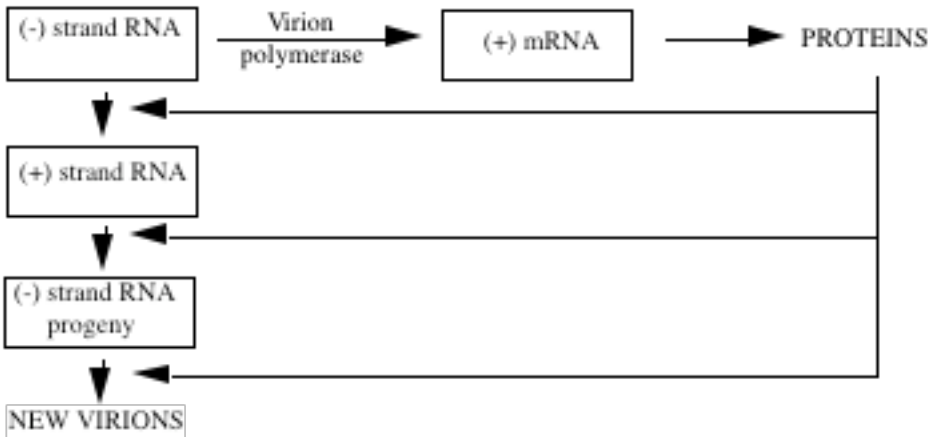
Rhabdo-	5 proteins; 11-15 kb	Bullet shape	Animals, plants	Fever, neurologic

- Genus: Lyssavirus (e.g., rabies virus)
- Genus: Vesiculovirus (e.g., vesicular stomatitis virus)

Borna-	5 proteins; 9 kb		Animals	Neurologic

- *similar to member of plant virus genus, Nucleorhabdovirus*

Generic mononegaviral replication scheme



Filoviridae

History/Outbreaks.

In 1967 simultaneous outbreaks of hemorrhagic fever occurred in Yugoslavia and in Germany, in lab workers who were processing kidneys from African green monkeys. There were 31 cases and 7 deaths. The virus was first characterized in **Marburg**, Germany and traced to a single shipment of Ugandan monkeys. Sporadic additional cases showed up in 1975, 1980, 1982 and 1987.

In 1976 there were epidemics of severe hemorrhagic fever in Zaire and Sudan. In Zaire, there were approximately 300 cases with an 80% fatality rate (due to Ebola-Zaire; EBO-Z). In Sudan, there were a roughly similar number of cases, with a fatality rate of roughly 50% (due to Ebola-Sudan; EBO-S).

As of 7 March 2003, there is an active outbreak on the Republic of the Congo. A total of 5 laboratory-confirmed and 105 probable cases, including 89 deaths have been reported (http://www.who.int/csr/don/2003_03_7/en/)..

Ebola virus was originally isolated in Zaire (now Democratic Republic of the Congo), and it was named after a small river in N.W. Zaire. Ultrastructurally the virus resembled Marburg virus but it was antigenically (and genetically) distinct. It now appears that at least three and probably four EBO viruses exist -- EBO-Z (Zaire), EBO-S (Sudan), EBO-CI (Côte d'Ivoire) and EBO-R (Reston). The first two are known to be highly lethal in humans and are spread via bodily fluids and by close (nonsexual) contact. The Reston virus *appears* to be less lethal in humans (0 deaths in 6 cases), although it is lethal in nonhuman primates.

Major outbreaks of Ebola occurred in 1995 in the Kikwit area of Zaire (over 315 cases, with 80% fatality; due to EBO-Z) and in the Gulu region of Uganda in 2000 (over 400 cases, but with roughly 50% fatality; due to EBO-S). It is uncertain how the Kikwit and Gulu outbreaks started. However, a smaller outbreak in 1996 in Gabon was traced to a group of 20 young Gabonese who trapped and caught a Chimpanzee that was sick. It is believed that exposure to Ebola occured during the preparation of the Chimpanzee, prior to cooking and consumption of the animal. Interestingly, Ebola was isolated only from meat-eating Chimps, and not from strictly vegetarian members of the same troupe of animals.

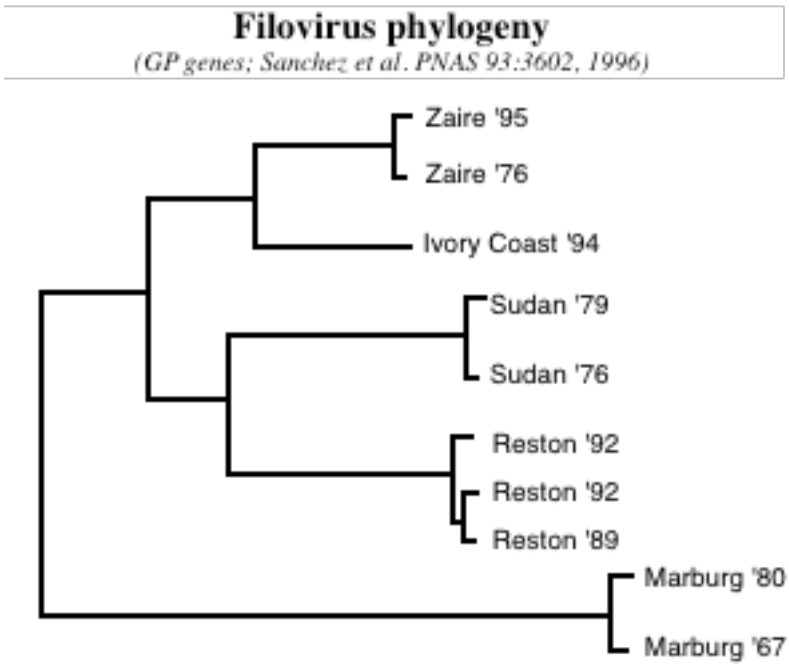
Outbreaks of EBO-Reston have occurred in US primate colonies in the Washington area (Reston, 1989) and in Texas (1990, 1996). These outbreaks were contained by destruction of all animals within the affected area of the facility. The outbreaks all appear to trace back to shipments of macaques from a single Philippine exporter. A total of 6 humans have become infected by EBO-Reston, but none has died.

Finally, while the major route of Ebola transmission is clearly close contact with bodily fluids and blood (eg, during health care, preparation for burial, etc), it is *possible* that some Ebola viruses *might* be transmissable via an aerosol route in some cases. One piece of evidence to support this idea is the fact that EBO-Zaire has been shown to infect rhesus monkeys that did not have direct contact with experimentally inoculated monkeys held in the same room *(Jaax et al. Lancet 346:1669, 1995)*.

Filoviruses are classic emerging infections. Filoviruses are Biosafety Level 4 agents (cf. HIV is only 2+). They are filamentous with a linear ~13-19kb genome. They can infect mice, hamsters, guinea pigs and monkeys -- although the **viral reservoir in the wild is not known**. Human epidemics seem to be related to blood-born nosocomial spread (often due to re-use of needles in hospitals; *nosocomial = hospital infection*) and to close contact with infected persons (since this is a hemorrhagic disease, this presumably would involve exposure to large amounts of blood). Primary infections with Marburg and Ebola are 25-90% fatal. Death is thought to be due to visceral organ necrosis (eg, liver) due to viral infection of tissue parenchymal cells. It is uncertain what role hemorrhage has in death.

Ebola virus vaccine. The first successful vaccination against this virus was reported in 1998, by Gary Nabel's group at the University of Michigan. In this report, a DNA vaccine encoding the Ebola virus glycoprotein was able to elicit a T-cell based immune response in guinea pigs, which was sufficient to protect the animals against infection with a live-Ebola virus (*Xu et al. Nature Medicine 4:37, 1998*). Subsequent studies in nonhuman primates have confirmed that a DNA vaccine can represent an important component of an effective Ebolav irus vaccine. Specifically, a combination of DNA immunization and boosting with adenovirus vectors encoding viral proteins resulted in the protection of cynomolgus macaques from an otherwise lethal dose of highly pathogenic, wild-type Ebola Zaire virus *(Sullivan et al. Nature 408:2000)*. This advance has led to the NIH (http://www.niaid.nih.gov/ttb/profile02.htm#ebola) forming a partnership with Crucell in May 2002 (www.crucell.com), to develop rAd-vectored Ebola virus vaccines, using Crucell's proprietary PER.C6 cell line for the propagation of E1-deleted rAd vectors (the major advantage of this cell line is that the E1 sequence it contains is smaller than the region that is missing from the E1-deleted adenovirus vectors; thus, it cannot recombine with the rAd vector and so there is no possibility of generating replication-competent adenovirus; RCA).

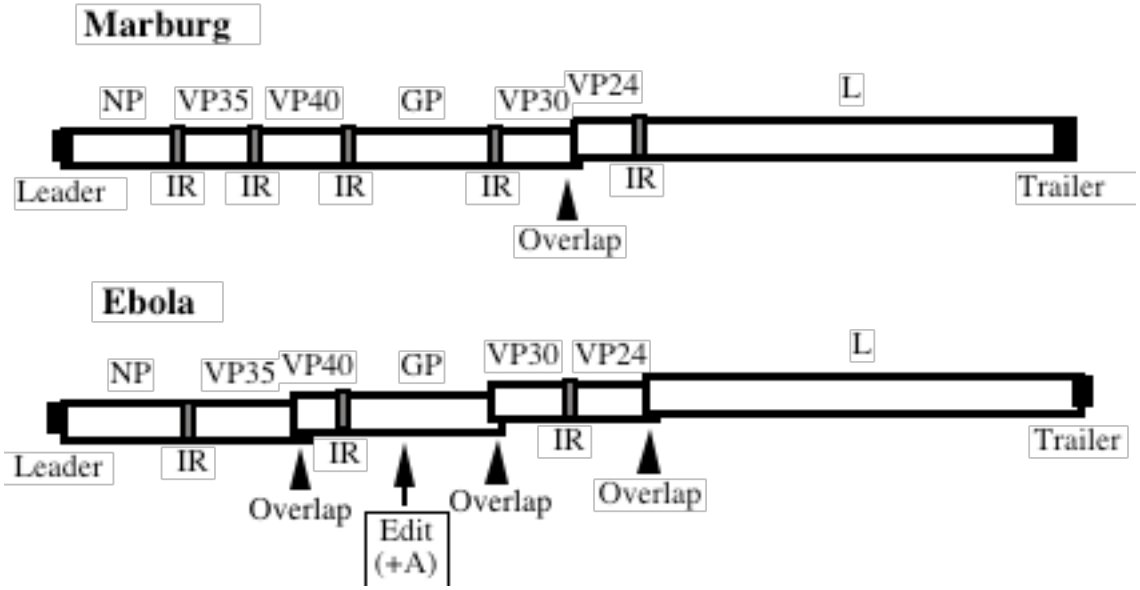
Filovirus genetics: Sequence analysis of Ebola viruses from outbreaks in 1976 and 1995 revealed a surprisingly high degree of genetic conservation for an RNA virus. One interpretation of this is that EBO viruses have coevolved with their natural reservoirs and do not change substantially in the wild (see below).



Overall, Filoviridae are more closely related to paramyxoviruses than to rhabdoviruses. Based on genetic analysis, two distinct groups identified (Marburg and Ebola). There is at least one important molecular difference between Marburg and Ebola -- in Marburg, the GP is encoded in a single open reading frame, while in Ebola, GP is encoded in two open reading frames. Expression of GP therefore involves a **site-specific RNA editing** event that is analogous to one which occurs in Measles virus. Specifically, a non-templated A residue is added to the mRNA, which allows joining of the two open reading frames. This results in the production of both a truncated, soluble form of the Ebola virus glycoprotein (sGP; 50-70 kD in size) and a full-length, transmembrane anchored version of the same protein (GP; 120-150 kD in size).

Filovirus genome structures

IR: intervening regions; GP: viral glycoprotein; VPxx: viral proteins; Editing site: addition of a nontemplated A



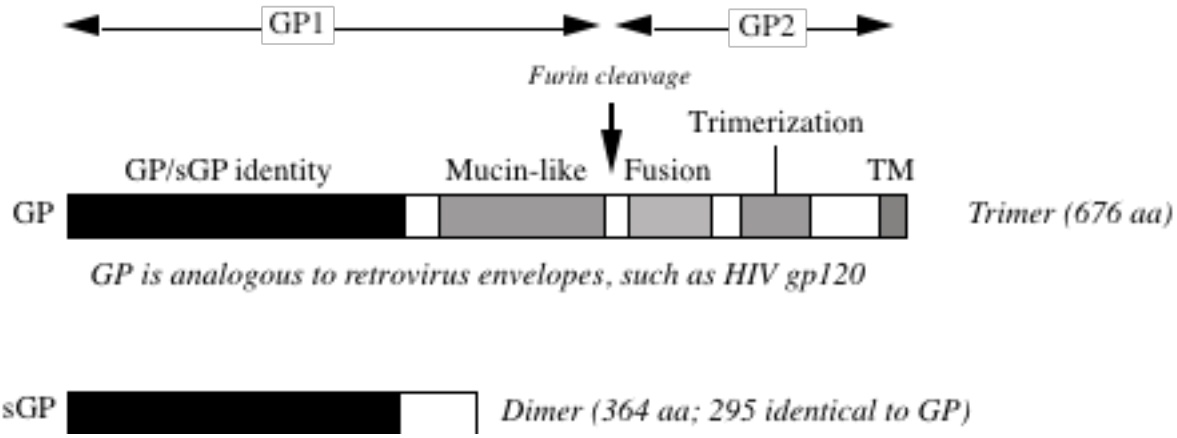
Ebolavirus sGP and GP have different functional properties, which may be important in disease pathogenesis. The functional subdomains of these molecules are shown below.

sGP: The soluble sGP molecule is secreted as a trimer, and is identical at its N-terminus to the homologous region of the transmembrane glycoprotein (GP). sGP interacts with neutrophils through CD16b, the neutrophil-specific form of the Fc receptor III, whereas the transmembrane glycoprotein (GP) interacts with endothelial cells but not with neutrophils *(Yang et al. Science 279:1034, 1998)*. It is possible that interaction of sGP with neutrophils results in the blockade of early events in the activation of these cells, thereby inhibiting inflammatory responses which might contribute to innate protection against viral infection. sGP may also act as a "decoy" for antiviral antibodies.

GP: The transmembrane glycoprotein is produced as a long precursor, which undergoes cleavage by a cellular protease (furin), to produce GP1 and GP2. Ebolavirus GP2 remains in the membrane (due to its transmembrane domain) and is responsible for mediating fusion between the virus and the plasma membrane, via its fusion domain. The GP1 component is attached to GP2 via a non-covalent linkage, and is thought to mediate virus attachment to its host cell(s), which include vascular endothelial cells.

Ebolavirus GP is also cytotoxic for vascular endothelial cells *in vitro*, and this is thought to contribute to the virus' ability to trigger vascular leakage (hemorrhage) *in vivo*.

Ebolavirus glycoproteins



Legend:

- GP: transmembrane glycoprotein (subsequently cleaved into GP1 and GP2 subunits)
- sGP: soluble glycoprotein
- GP/sGP identity: region shared by sGP, GP
- Mucin-like domain: highly glycosylated domain of GP that is essential for cytotoxicity
- Fusion domain: responsible for membrane fusion; located within GP2
- Trimerization domain: allows GP2 to form stable trimers, like other viral fusion proteins
- TM: transmembrane domain: anchors GP2 in the membrane

BORNA DISEASE VIRUS

Pathogenesis: Borna disease virus (BDV) is a **neurotropic** agent that naturally infects horses and sheep, and which is capable of infecting primates. The disease induced by BDV resembles neuropsychiatric illnesses (*schizophrenia*). It is uncertain whether the virus has any relationship to human neurologic disease.

RHABDOVIRIDAE

Greek "rhadbo": rod-shaped

Over 100 rhadboviruses exist & they infect almost all animals. Two genera affect mammals:

Genus	Features	Example
Lyssa-	invade CNS; *(fr. Greek "lyssa": frenzy)*	Rabies virus
Vesiculo-	invade epithelial cells (usu. tongue) & cause vesicles	Vesicular stomatitis virus (VSV)

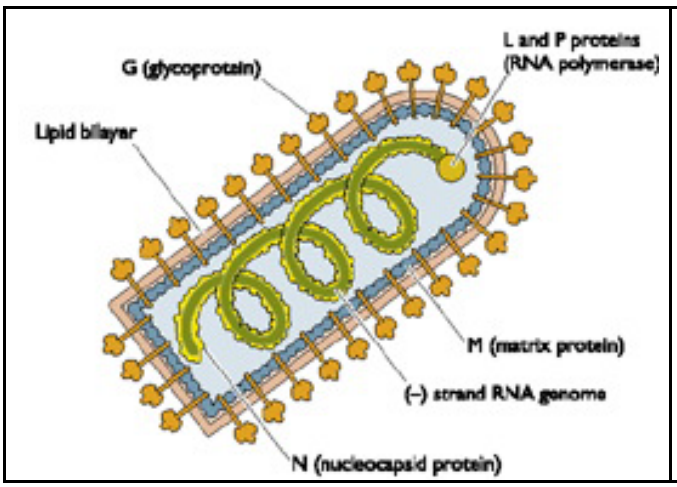
Rabies: Causes encephalitis in animals and in humans they bite**.** Rabies virus can infect all warm-blooded animals, but some are more susceptible than others (eg, foxes, wolves > dogs, skunks, raccoons > opposum). It is spread to humans via animal bites. In the US it is most prevalent in skunks, but also found in raccoons and sometimes in bats. Elsewhere rabies is more common in humans, due to its presence in dogs.

Bats and rabies: In the U.S. about 1-2 cases of rabies occur each year. Since 1990, 20 of 22 domestically acquired human rabies infections in the United States have resulted from infection with bat rabies variants, and in only one of these cases was there a clearly documented bat bite (http://www.wadsworth.org/rabies/bat.htm). Many of these bat rabies strains were silver-haired bat (SHB) rabies virus. SHBRV is carried both by silver-haired bats (relatively rare and solitary) and also by other strains of bats *(overall, much less than 1% of all bats test positive for rabies, and the bats most often found around humans -- brown bats -- have never been shown to have cause human disease)*. The rarity of SHBRV is strongly suggestive that something unusual is going on here. In addition, data show that the SHBRV variant replicates with unusually high efficiency in cultured epithelial cells, particularly at low temperatures (34°C). This may allow the virus to replicate more efficiently in the skin *(Morimoto et al. PNAS 93:5653, 1996)*.

CLINICAL PICTURE: Whether disease results reflects the location and severity of bites (typically ~15% rate of infection). Disease onset is slow, with an unusually variable incubation period (can be over a year) during which virus replicates in muscle near the entry site. Thereafter, the virus enters peripheral nerves. From here, it travels to spinal ganglia & enters the brain. It is then disseminated to all tissues (including salivary gland). Death is inevitable if the virus enters nerves, but post-exposure intervention before this is generally successful.

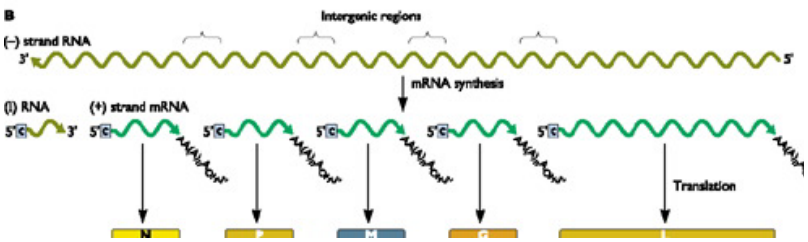
Control of rabies: is achieved by controlling its animal reservoir -- ie, by vaccinating domestic animals and also by the use of vaccine-containing bait to target wild animals. For exposed humans, there is a vaccine.

VSV: Causes epidemic but self-limiting vesicular disease of cattle. Also infects swine, horses, humans & even insects (**very broad host range**). In humans, it causes a mild flu-like illness that's fairly common in lab workers. In keeping with its broad host range, the VSV receptor is not a protein (prolonged trypsinization of cultured cells doesn't block infection). It may be phosphatidyl serine.



VSV Virion (left) and Genome (below)

Flint et al., Appendix Fig 5 (p757)



Molecular Biology of VSV

Overall, the molecular biology of VSV is considerably better understood than that of rabies virus

The morphology and structure of VSV is similar to that of rabies virus. The particles are bullet-shaped and are composed of two major structures -- a **nucleocapsid** or ribonucleoprotein (RNP) core and a lipoprotein **envelope** which surrounds that core.

VIRAL RNP CORE: The nucleocapsid or RNP core is the infectious component of VSV and all other rhadboviruses. As shown in the diagram, this core includes the viral genomic **RNA** which is tightly associated with the highly abundant **nucleocapsid protein (N)**. The RNP core also contains less abundant proteins -- the **phosphoprotein (P)**, and the **viral RNA polymerase (L)**.

N protein: The function of the N protein appears to be (1) to promote RNA encapsidation or packaging and (2) to allow genome replication, by favoring antitermination of transcription (ie, by allowing the viral polymerase to read-through the stop/start signals located between the viral genes).

L protein: This is the viral RNA-directed RNA polymerase. It is not active on its own, however, since P protein is needed for catalytic activity.

VIRAL ENVELOPE: The major components of the VSV envelope are (1) the membrane-anchored viral **glycoprotein (G)** and (2) the **matrix protein (M)**. Roughly equivalent amounts of the two protein are found in each virion (approx. 1500 molecules per virion).

G protein: The glycoprotein, G, forms trimeric spikes on the surface of the viral particle and it forms both the major antigenic determinant on the virus, as well as the major receptor-binding molecule on the virus. G protein undergoes a conformational shift at mildly acidic pH (< 6.0), which stabilizes the trimer and exposes a hydrophobic domain that can insert into cellular membranes and allow membrane fusion to occur. Thus, VSV fusion is activated in the endocytic vesicle, in response to acidic pH.

Viral gene expression

After entry into its host cell, and uncoating of the RNP core, VSV begins to express its genes. Since the viral genome is of negative sense (ie, of opposite polarity to mRNA), the very first step is transcription of viral mRNAs. *Note that the RNP core is transcriptionally active, and that is not inhibited by actinomycin-D (unlike cellular RNA polymerases, which use a DNA template to direct mRNA synthesis).*

Viral transcription begins at the 3' end of the viral genome, at a **single promoter element**, and proceeds sequentially across the genome. It is generally believed that the individual gene-unit-length mRNAs are produced by a **stop-start** trancription mechanism (see below). One result of this is that the transcriptase pauses and transcription is attenuated about 30% at each gene junction. This in turn produces a gradient of mRNA production, such that N>P>M>G>L.

Stop/start transcription is acheived by the presence of transcriptional signals at gene boundaries. There is a 5'-initiation signal, as well as 3'- polyA and termination signals, which are ordered: [polyAsignal/terminator]--[intergenic region]--[initiator]. *Note that the intergenic region (2 nucleotides) is not transcribed during viral mRNA synthesis.*

Viral RNA replication

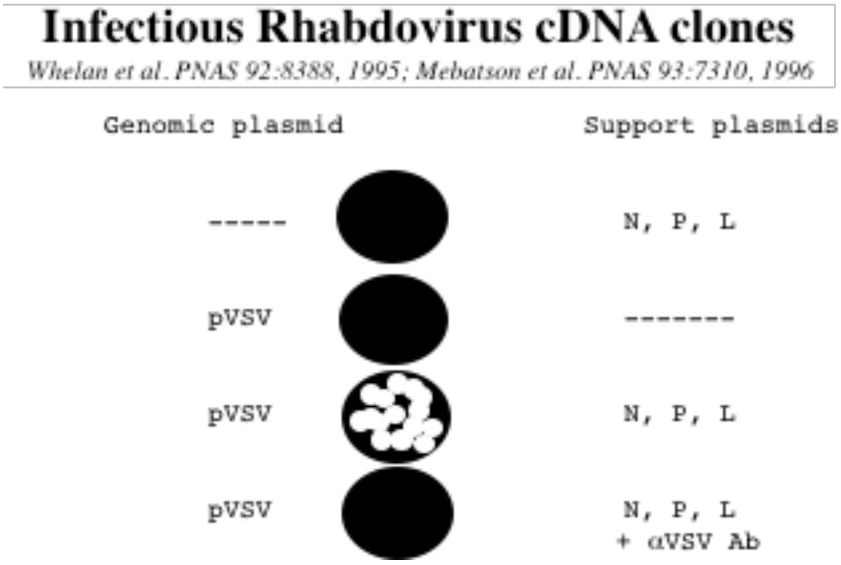
Unlike viral mRNA transcription, viral RNA replication requires the virus to form a single complete copy of its genome. Thus, replication differs from mRNA transcription in that transcriptional "start/stop" signals must be ignored somehow.

The decision to replicate the viral genome must therefore be made when the first intergenic region is encountered (this is located between the region that encodes the short untranslated leader RNA and the gene encoding the N-protein). This **intergenic region must be read-through** in order for viral RNA replication to occur.

Interestingly, viral RNA replication **requires active translation** (this was proved experimentally, since viral RNA replication, but not viral mRNA synthesis, was blocked by inhibition of protein synthesis using cycloheximide). This observation is consistent with a model in which newly formed viral N protein selectively binds to the viral leader RNA. By doing so, N prevents the recognition of transcriptional termination signals. Thus, the switch from mRNA synthesis to RNA replication is regulated principally by the **anti-termination activity of the N protein**.

Molecular Genetics and Vectors

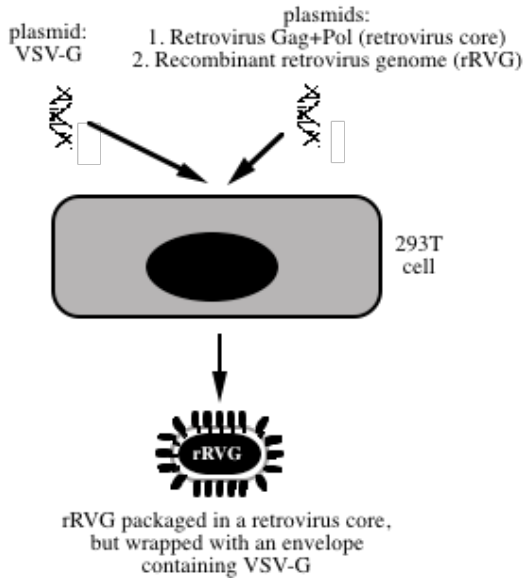
Replication of VSV has been difficult to study using recombinant DNA methods. This is because deproteinized RNA is not infectious. Likewise, RNA transcribed from cDNA clones is not by itself competent to initiate infection. The virion RNA-polymerase is needed for infectivity, and in addition the RNA must be encapsidated to be a functional template for the polymerase. Finally, constant synthesis of N protein is needed for replication. Recently, great progress has been made in this area. Methods for the production of infectious virus from cDNA clones of both VSV and Rabies virus have been developed. This is allowing the development of novel vector systems based on VSV and Rabies virus.



KEY: *The dark circles represent cell monolayers, and the white areas are plaques in these monolayers, which were caused by infectious virus. You can see that infectious virus was produced ONLY when an intact cDNA copy of the VSV genome was introduced into cells together with support plasmids encoding the viral RNP constituents (ie, N, P and L). The bottom panel proves that the plaques seen were due to VSV, since plaque formation was blocked by the addition of a neutralizing antiserum directed against VSV (anti-VSV Ab).*

VSV G-protein is widely used to generate **retrovirus pseudotypes**. Pseudotypes are the result of phenotypic mixing of different viruses, and contain a core (and genome) from one virus, combined with the envelope (and receptor-binding activity) of the second virus.

VSV-G Pseudotyped Retrovirus Vectors



In general, pseudotypes can be generated only between fairly closely related viruses (such as VSV and Ebola virus), but exceptions exist. One notable exception are retroviruses. It has long been known that VSV can generate pseudotypes with a variety of retroviruses, including HIV-1.

Retrovirus pseudotypes bearing the VSV G-protein in place of the natural retrovirus envelope have several features which make them useful for gene therapy. These include:

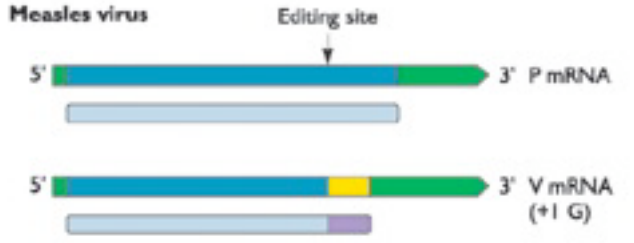
1. Extended host cell range. The VSV G-protein allows one to deliver the retrovirus "payload" (i.e., the recombinant genome) to a wide array of mammalian & animal cells, including fish. It also allows one to deliver genes to certain human cell types, such as hematopoietic progenitor cells, which are otherwise difficult to target.
2. Increased physical stability. The VSV G-protein is much more stable than the natural retrovirus envelope. This allows one to concentrate the viral particles, and to generate high-titer stocks which are more useful for gene transfer.

PARAMYXOVIRIDAE

Chua et al. Science 288:1432, 2000; Goh et al. N. Engl. J. Med. 342:1229, 2000 (Nipah)
Wang et al. J. Virol. 74:9972, 2000 (Hendra virus)

Subfamily **Paramyxo- virinae (Genera: Paramyxo-, Morbilli-, Rubula-)**
Respiro-: eg, Parainfluenza viruses 1 and 3: have H and N (single molecule: HN)
Rubula-: eg, mumps virus: have H and N (single molecule: HN); has extra gene (SH)
Morbilli-: eg, measles virus: have H but no N
Henipa-: eg, Nipah, Hendra viruses: have H but no N *(this genus has been proposed but is not yet official)*
Subfamily: **Pneumo- virinae (Genus: Pneumovirus)**
Pneumo-: eg, respiratory syncytial virus (RSV): has neither H nor N; more divergent

RNA editing during Measles virus mRNA synthesis
The P gene mRNA of Measles virus is cotranscriptionally edited at a specific site. This **RNA editing** event involves the addition of a nontemplated G residue at this position during mRNA synthesis *(this occurs because the RNA polymerase slips or "stutters" when it encounters a run of C residues at this site on the template RNA strand)*



MV RNA Editing
Flint et al. Fig 10.13B (p352)

The extra G introduced at the editing site results in a change in the translational reading frame, to one which contains a translational stop codon. Thus, the edited RNA encodes the **V protein**, which is identical to the P protein at its N-terminus, but different at its C-terminus

Measles Virus: Envelope proteins
All paramyxoviridae possess two membrane or envelope proteins. One is involved in cell **attachment** and the other mediates **fusion** with the host cell membrane, in a pH-independent manner.

Attachment Protein. The attachment proteins of the paramyxovirinae bind to sialic-acid containing receptors on cells, and these viruses are therefore able to agglutinate red blood cells (hemagglutination). In the case of viruses in the genera respirovirus and rubulavirus, these viral hemagglutinins also possess neuraminidase activity and are thus refered to as **HN proteins** (hemagglutinin-neuraminidase). In the case of viruses in the genus morbillivirus, the hemagglutinin lacks neuraminidase activity (thus, **measles virus encodes an H protein** and not an HN protein).

Role of Neuraminidase. It is believed that neuraminidase prevents aggregation of viral particles to the plasma membrane during viral budding, and thus facilitates virus release from infected cells. This means that neuraminidase must be inhibited during the early steps of virus entry and that it must become activated during the late stages of virus exit. This may occur in part because the activities of hemagglutinin and neuraminidase are regulated by pH and by halide ion concentration. Specifically, the pH and halide ion concentration of the extracellular environment is optimal for hemagglutination, while neuraminidases function best at acidic pH (such as can be found within the Golgi network inside cells).

Note that measles virus (MV) does not in fact use sialic acid as its receptor -- presumably because its H protein has a relatively low affinity for sialic acid. As a result, MV does not need a neuraminidase. In stead, **measles virus H protein is thought to bind to a specific receptor, SLAM** *(signalling lymphocyte-activation molecule; CDw150). SLAM is present on some T cells and B cells (Tatsuo et al. Nature 406:893, 2000).*

The Pneumovirus, respiratory syncytial virus (**RSV**) does not hemagglutinate and its receptor is unknown. In this case, viral attachment to the host cell is mediated by the **G (glycoprotein) protein**.

*Note that some highly passaged vaccine strains of MV bind to **CD46** -- a molecule which is a member of the immunoglobulin gene superfamily; clinical isolates of MV do not, however, bind CD46.*

Paramyxovirus replication

In general terms, paramyxovirus replication is broadly similar to that of rhabdoviruses.

One important and unique feature of **measles virus infection**, in particular, is the virus' ability to persistently infect brain cells, which has been implicated in SSPE. Persistent MV infection appears to involve at least two methods for **specific attenuation of M gene expression**. First, specific downregulation of M gene expression can occur as the result of inefficient transcriptional termination and polyadenylation at the upstream ORF (which is expressed normally). Second, biased hypermutation of the M gene region has been described. This is due to the action of a cellular enzyme that converts adenosines to inosines in dsRNA (double-stranded-RNA-adenosine deaminase, dsRAD). *Note that dsRAD is also responsible for site-specific editing of Hepatitis Delta virus RNA.*

Pathogenesis of MV infection

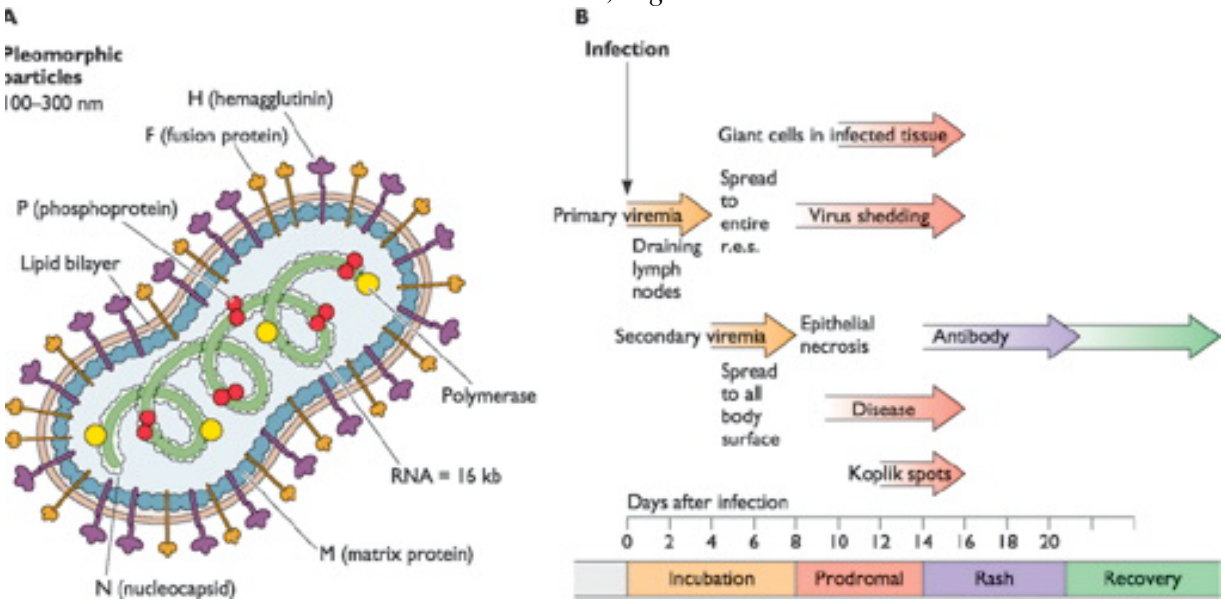
MV is a classic emerging pathogen. MV is a relatively new disease of humans which evolved from an animal morbillivirus (MV most closely resembles a pathogen of cattle known as rinderpest virus). Measles was first described in the tenth century. Since it causes a highly contagious acute infection which results in lifelong immunity, and it has no animal reservoir, it requires an urban setting to survive (200,000+ people). Cities this size first emerged ~3000 BC in Egypt and Sumeria. This is likely when measles and mumps emerged. These cities remained isolated until trade began. Epidemics of disease (measles, smallpox) then began around 200-400 AD. Measles was carried to the New World by Europeans and caused many deaths among native Americans, who had not encountered MV previously.

MV is a major killer of the world's children. Worldwide, measles kills almost 800,000 million children per year (http://www.unicef.org/measles/disease.htm). As a result, WHO and UNICEF are working hard to further raise immunization rates in all villages and cities, including poor areas. Ultimately, the WHO and UNICEF intend to eradicate measles completely. This is possible because (1) there is no animal reservoir for MV, (2) there is only one serotype of the virus, (3) most cases are clinically identifiable and (4) an effective live-attenuated vaccine is available.

Pathogenesis. Measles is a childhood infection that is spread by a respiratory route. Following infection, the virus replicates in lymphoid tissues. It then enters the blood (viremia) and spreads through the body, reaching its target tissues (principally, the lungs), where replication occurs. This initial period of infection is asymptomatic and lasts about 10-14 days, at the end of which clinical signs of disease become apparent -- notably, fever, cough and conjunctivitis. Roughly 2-3 days after the onset of these symptoms, the characteristic measles rash appears. This coincides with the appearance of an antiviral immune response. Recovery results in lifelong immunity to infection.

Measles Pathogenesis

Flint et al., Fig 15-10



Immune responses. Recovery from MV infection is mediated in large part by a cellular immune response to the virus (cytotoxic T cells, CTLs). However, one of the striking features of MV is its ability to cause **immune suppression** *in vivo* and *in vitro*. Production of cellular responses to new antigens is significantly inhibited, which predisposes MV infected children to concurrent infection by other pathogens (such as bacteria, which can cause fatal pneumonia in MV-infected children). The mechanism(s) by which MV causes immune suppression is believed to involve virus infection of dendritic cells (which are involved in antigen presentation) as well as infection of other immune cells. **MV infection of dendritic cells** (DC) leads to apoptosis, and to inhibition of the ability of DCs to stimulate T cell proliferation (which is important for the generation of immune responses.

Autoimmunity. In addition to immune suppression, MV infection can be associated with autoimmunity. Specifically, an autoimmune demyelinating disease, **postinfectious encephalomyelitis** (PIE) is an important complication of measles which occurs within 14-28 days of infection in about 1 in 1000 cases. It is associated with an immune response to myelin basic protein. It is not clear how this autoimmunity is initiated, although it is possible that MV antigens may resemble myelin (molecular mimicry). Thus, an anti-MV immune response may lead to attack on a self antigen -- myelin.

Persistent infection. MV can establish a persistent infection in brain cells *in vitro* and *in vivo*. As noted above, this is often associated with suppression of expression of the viral M protein. Persistent MV infection of the brain can be associated with a very rare disease, Subacute Sclerosing PanEncephalitis (SSPE), which occurs several years after initial MV infection in about 1 in 1,000,000 children.

Clinical features and complications of measles. Serious symptoms of measles include:

(1) **Respiratory disease**. Pneumonia can be caused by MV itself (giant cell pneumonia), particularly in immune suppressed persons OR (more commonly) by secondary bacterial or viral infections.

(2) **Gastrointestinal disease**. Diarrhea is a very common complication of measles. This can be a major problem in children who are already malnourished or at risk for malnutrition. Also, the severity of MV infection has been shown to be much worse in children who are deficient for **vitamin A**. *This is one of the major reasons why WHO and UNICEF support vitamin A supplementation programs in the developing world (vitamin A can be administered very cheaply in megadose capsules).*

(3) **Neurologic disease**. Postinfectious encephalomyelitis (PIE) occurs in about 1 in 1,000 cases, usually within 14-28 days of infection. Slowly progressive neurologic disease can occur in immunosuppressed persons (measles inclusion body encephalitis, or MIBE) and SSPE occurs at a very low frequency in immunologically normal children, usually several years after initial MV infection.

(4) **Eye disease.** MV is an important cause of blindness due to corneal lesions. Again, vitamin A deficiency has also been implicated in blindness, and may exacerbate MV induced eye damage.

(5) **Atypical measles.** A severe form of measles, with more prolonged fever, worse skin lesions and more serious pneumonitis, has been observed in individuals who received the inactivated MV vaccine used in the US from 1963 - 1967. This formalin-inactivated vaccine provoked an unbalanced immune response, with high level antibodies to the viral H protein but little reactivity to the F or N proteins.

Other paramyxoviruses

Respiratory syncytial virus (RSV). RSV infects infants from **6 weeks to 6 months of age** and causes 90,000 hospitalizations and **4500 deaths each year in the US**. It usually causes upper respiratory infection, but in 25-40% of cases, lower respiratory symptoms occur. *RSV is the most important viral cause of lower respiratory disease in infants and children.* In the elderly, severe pneumonia can occur. RSV is a major cause of nosocomial (hospital) infections. Aerosolized ribavirin can be helpful, as can passive antibodies (RSV immune globulin). Two antigenic subtypes exist, but there is no effective vaccine, in part because (1) reinfection is common; (2) immunity is incomplete (infection occurs in infants, despite the presence of maternal antibodies).

ParaInfluenza viruses. PIV-1, 2 and 3 are second only to RSV as causes of serious respiratory tract disease in infants and children. **Mumps virus.** Mumps usually causes a benign systemic febrile illness with swelling of salivary glands. It can, however, infect the CNS and can cause meningitis, encephalitis, deafness plus orchitis. A live attenuated vaccine has been used in the US since 1967; mumps is now rare.

New and emergent paramyxoviruses

A recent example of an emerging morbillivirus infection occured in Australia in 1994. 14 horses died as a result of infection with **Hendra virus** in Queensland. Two people who had close contact with these horses also became infected and one developed a fatal respiratory illness. A second fatal case of human Hendra virus infection was subsequently described in another region of Queensland, some 800 kilometers away from

the first outbreak. Followup studies have revealed that Hendra virus infection of horses is rare. However, the virus has been found in numerous bats -- suggesting that these animals may be the virus' natural host. *Note that Hendra virus was initially known as equine morbillivirus, but subsequently renamed to reflect its somewhat distant genetic relationship to the morbilliviruses.*

Research into Hendra revealed two previously unknown diseases associated with bats in Australia (http://www.csiro.au/page.asp?type=faq&id=HendraVirus). The **Australian bat lyssavirus** was identified in 1996, and is closely related to the rabies virus; it has been associated with at least two human fatalities. The **Menangle virus** was isolated in 1997 from pigs, and has been associated with a flu-like illness in humans; it is thought that Menangle is a member of the *Rubulavirus* genus. Like Hendra virus, Menangle virus is also carried by fruit bats.

The isolation of the Hendra virus in 1994, and the Menangle virus in 1997, presaged the identification of **Nipah virus** in 1999. Nipah was isolated from pigs and from humans with encephalitis in Malaysia; it was a spread by a respiratory route through the pig population and caused the death of over 100 Malaysians from encephalitis. All those killed had close contact with pigs (pig farmers and workers) and the outbreak was contained by slaughter of one million pigs. Like Menangle, it is thought that Nipah was transmitted initially by bats.

Pigs are thought to have played a crucial role in the emergence of Nipah, since these mammals are unique in being maintained in very high concentrations, in situations where epidemic disease can easily occur. It has been suggested that Nipah was able to initially establish itself in these pigs, following transmission from fruit bats, and that the virus was able to become adapted to mammals (pigs) over the course of perhaps a couple of years in Malaysian pig farms. Infection of the pigs then gave the virus ready access to humans (pig farmers, pig workers). The linkage of the Nipah virus outbreak to livestock production has important implications, and highlights the potential risks that can be associated with the high intensity farming of hogs and chicken, in particular.

The Nipah and Hendra viruses are genetically related more closely related to each other (70-78% nucleotide homology) than to any other member of the family *Paramyxoviridae* (maximum of 49% homology to any other virus in this family). Furthermore, the genomes of Nipah and Hendra viruses are considerably larger than the other *Paramyxoviridae* (> 18 kb, compared to 15-16 kb for the other family members). Nipah and Hendra also share a number of other unique genetic features which mark them as separate members of the *Paramyxoviridae*. As a result, it has been proposed that these viruses be classified into a new genus in the family *Paramyxoviridae; a proposed new for this group of viruses is the Henipavirus Genus (Wang et al. J. Virol. 74:9972, 2000).*

Nipah and Hendra also share the common feature of being **zoonotic paramyxoviruses** with an expanded host range that includes humans and other animals (horses, pigs); both are thought to be transmitted from a natural reservoir in **fruit bats**.

Phylogenetic analysis of Nipah and Hendra viruses