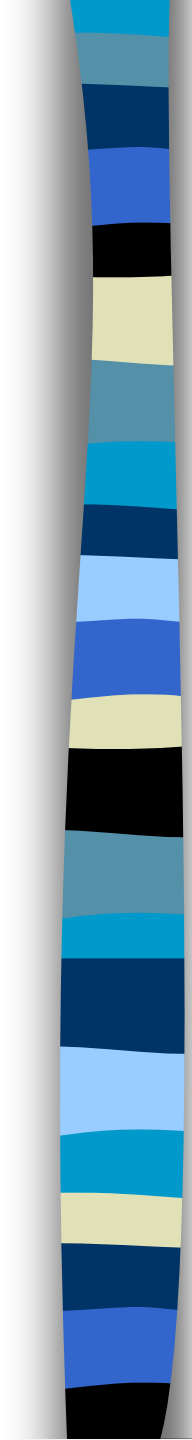


Overview of Emerging Pathogens

**Peter Simmonds
Roslin Institute
University of Edinburgh
UK**





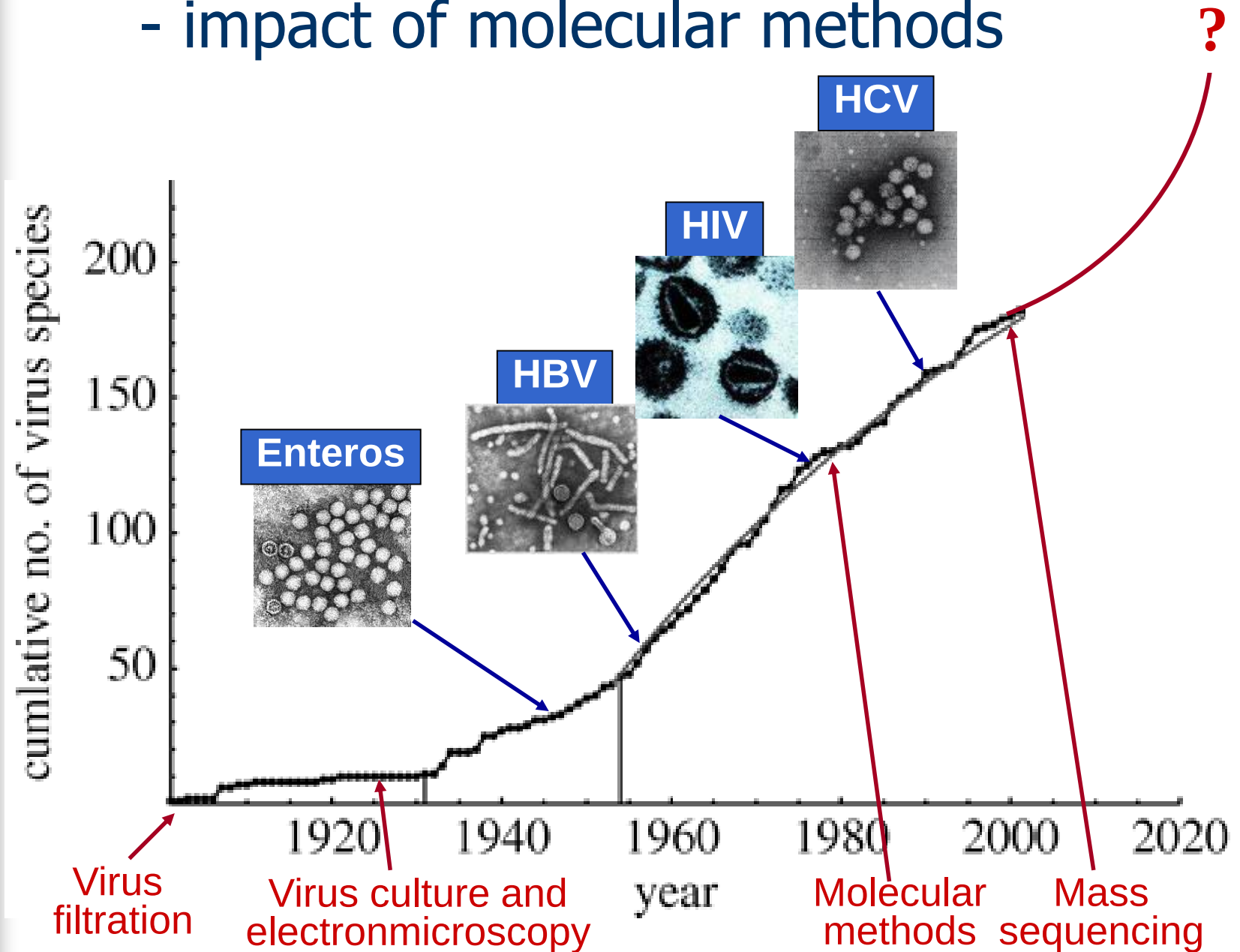
Emerging viruses

- Appearance of new infections in humans
 - Increase in human population density
 - Long-range migration and frequent travel
 - Movement of infected individuals within incubation period of virus
 - Encroachments on environment, changing farming practices facilitates zoonotic transfer

- Improved technologies
 - Agnostic, deep sequencing methods
 - May read most/all RNA/DNA sequences in a sample
 - Continued improvements in technology
 - Increased read lengths and accuracy
 - Declining costs
 - Identification of a large number of human, veterinary, plant and insect viruses
 - Scope for large scale environmental screening

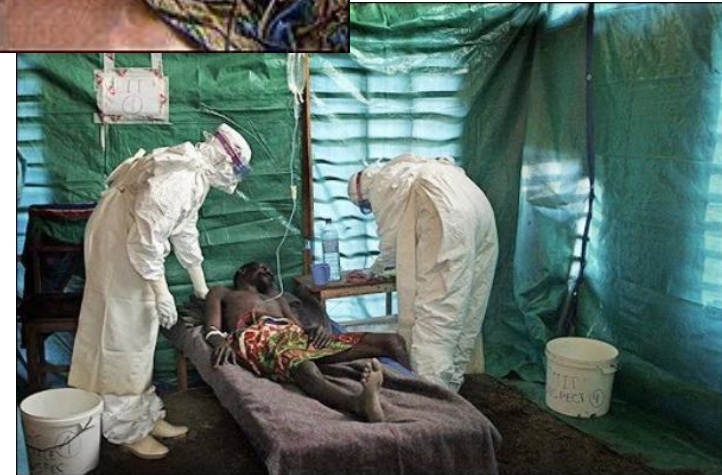
Virus discovery

- impact of molecular methods



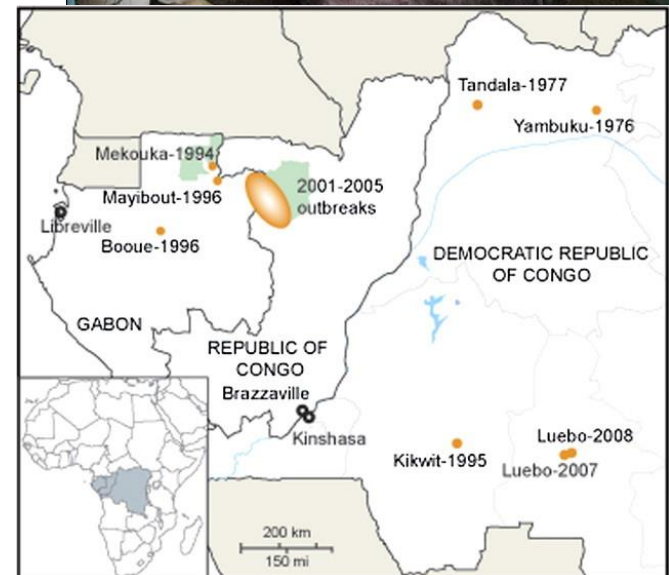
Ebola virus

- Highly pathogenic virus
 - 78% case / fatality rate
 - Infects the gastrointestinal tract, may cause haemorrhagic disease
 - Highly contagious



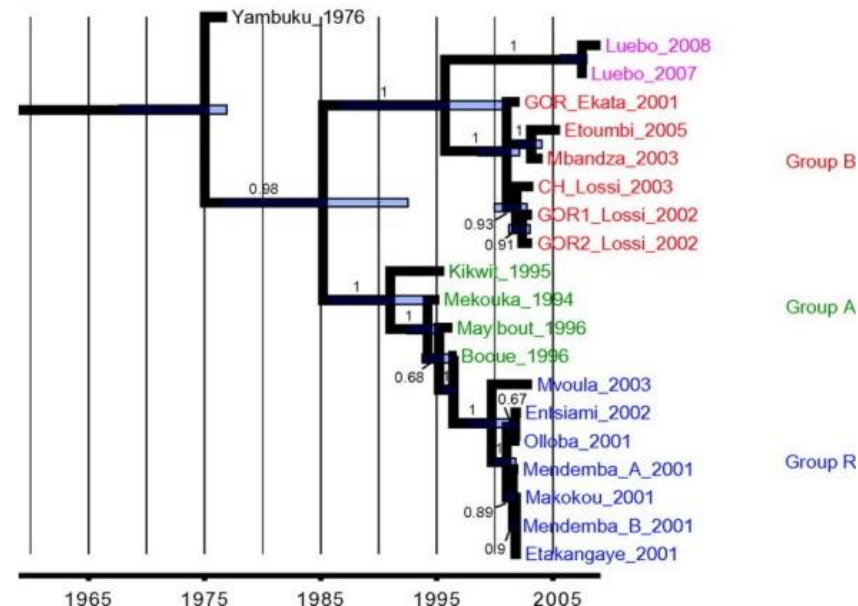
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 - Rare, highly sporadic occurrence
 - Limited to remote regions of Central Africa
 - 4 major outbreaks (1976-7, 1994-6, 2001-5, 2007-8)



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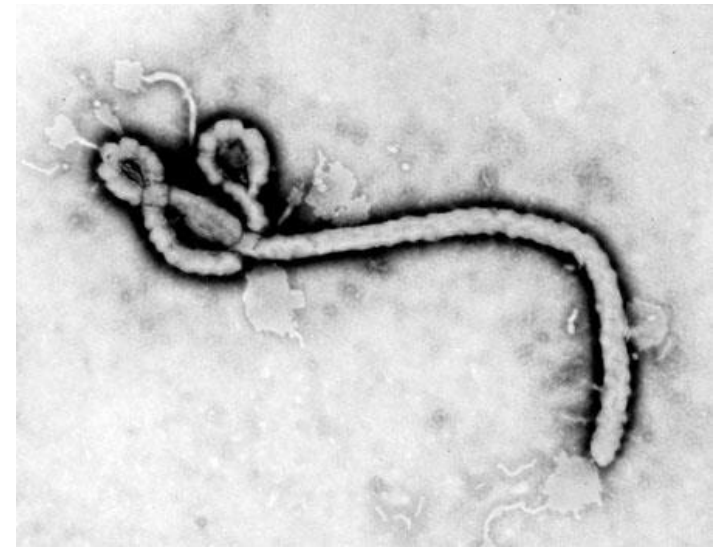
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 - 4 major outbreaks (1976-7, 1994-6, 2001-5, 2007-8)
 - Separate evolutionary lineages for each outbreak
- Zoonotic source of infection suspected
 - Cases after contact with bats
 - Widespread, asymptomatic carriage detected in chiropteran bats (*H. monstrosus*, *E. franqueti* and *Myonycteris torquata*)



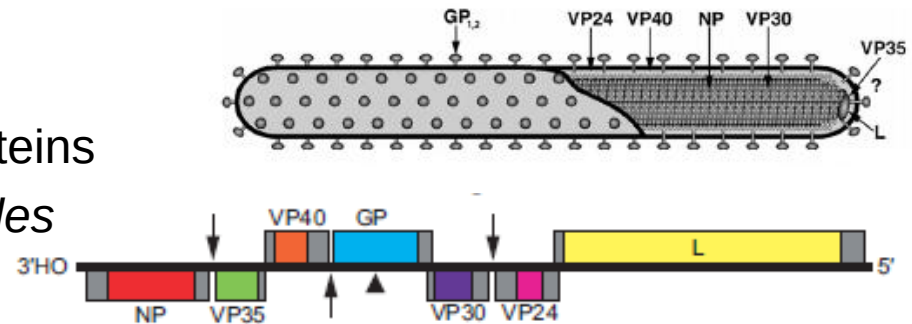
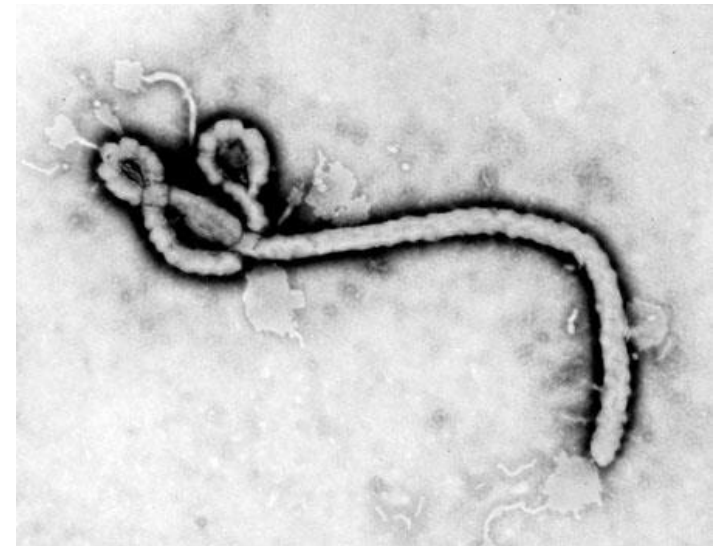
Filoviridae

- Family of large, negative-stranded RNA viruses
 - Enveloped
 - Thread-like appearance



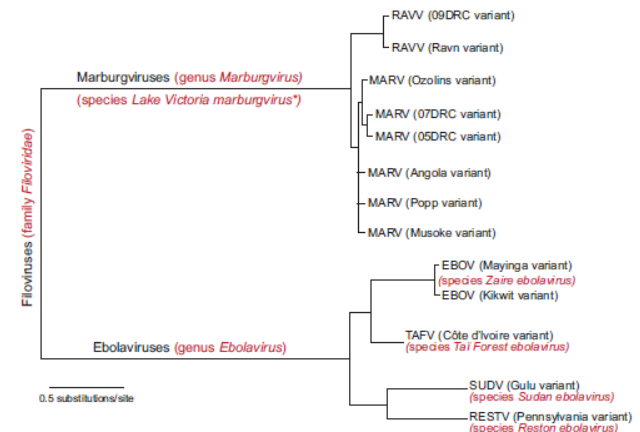
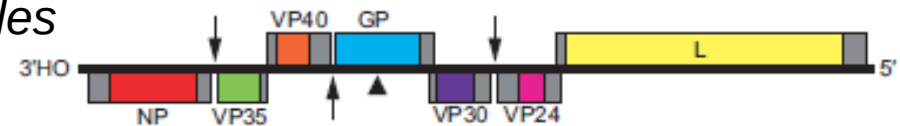
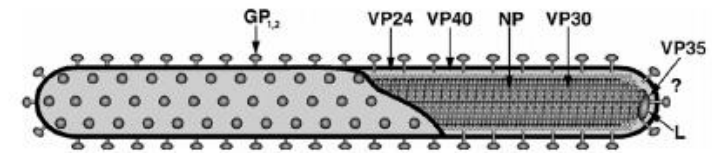
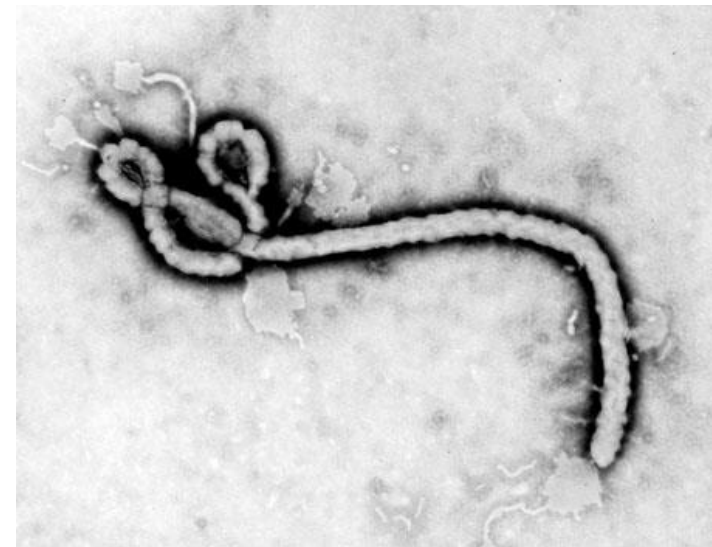
Filoviridae

- Family of large, negative-stranded RNA viruses
 - Enveloped
 - Thread-like appearance
- Genome organisation
 - Conserved replication proteins
 - Member of *Mononegavirales*



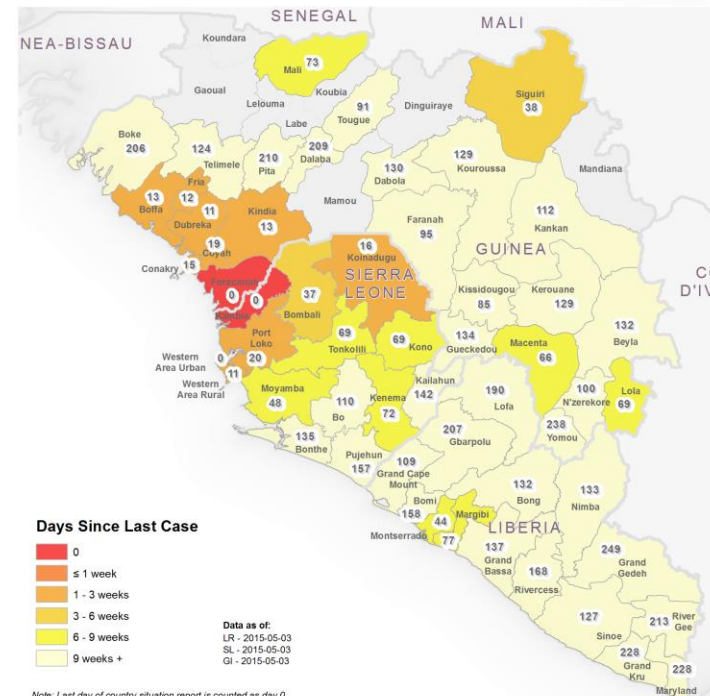
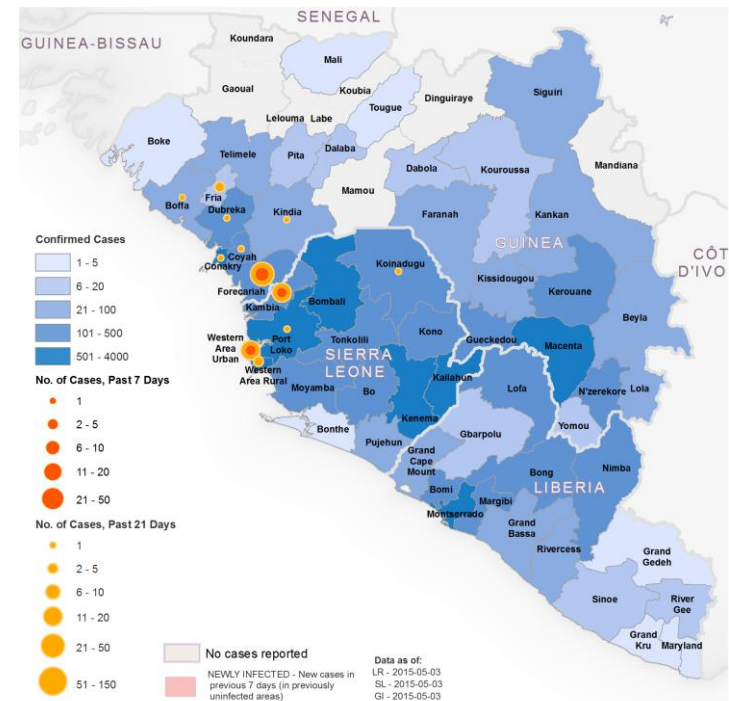
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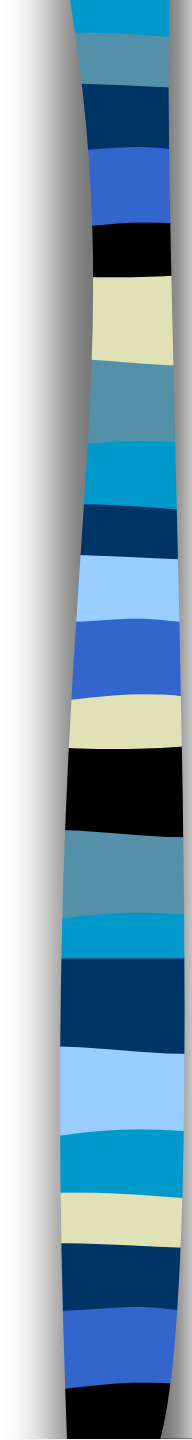
- Family of large, negative-stranded RNA viruses
 - Enveloped
 - Thread-like appearance
- Genome organisation
 - Conserved replication proteins
 - Member of *Mononegavirales*
- Two currently assigned species
 - Similar circulation in bats
 - Zoonotic spread with variable disease outcomes



2014/15 Outbreak

- Largest outbreak to date
 - 2240 cases and 1229 deaths in mid-August, 2014
 - 26,628 cases and 11,020 deaths in early May, 2015
- Focus on West Africa; zoonotic source unknown
- Sustained human-to-human transmission
- Can be contained,
 - Patient isolation, curfews
 - Improved ITU facilities, WHO response
 - Limited spread elsewhere (USA, UK, Spain, Nigeria)
- Treatment and prevention
 - ZMapp, 3 monoclonal antibodies
 - Urgent scale up of production
 - Vaccine development and evaluation



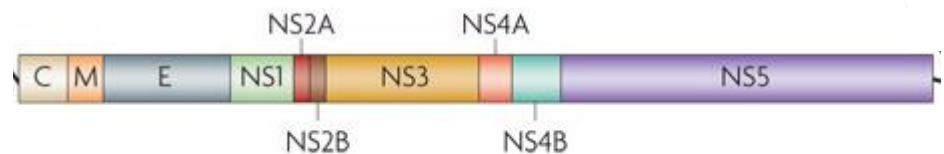
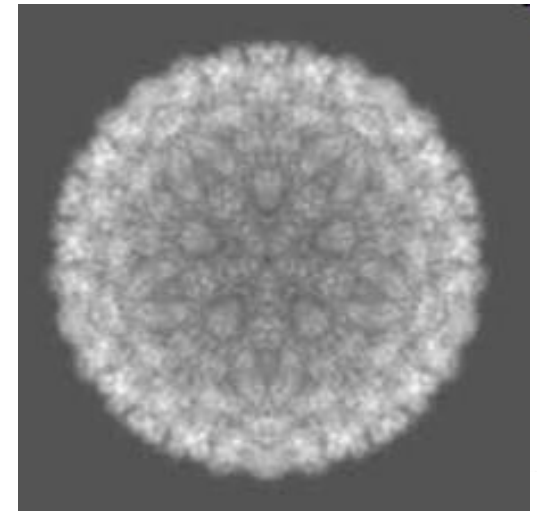
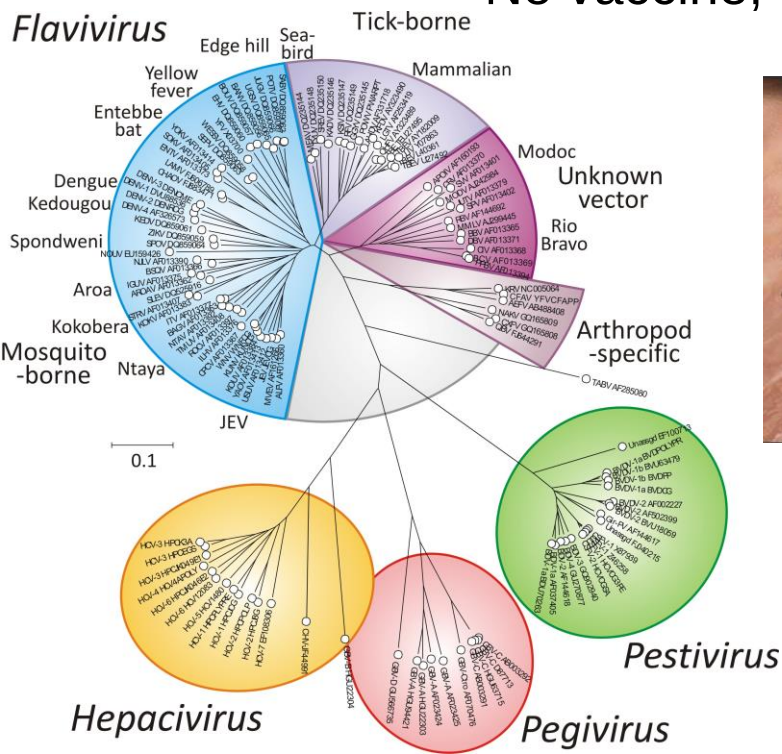


Emerging viruses and blood transfusion

- Emerging and re-emerging viruses
 - Change in incidence / epidemiology
 - Hepatitis E virus, HAV, Ebola virus
 - Expansion in geographical range of vector
 - Dengue viruses, Chikungunya
 - Transmission by long distance travel
 - West Nile virus, Chikungunya, Zikah virus
- Newly discovered blood-borne viruses
 - Identified by new technology
 - HCV, human pegivirus,
 - TTV
 - GBMV
 - Newly spread into human populations
 - HIV-1
 - Severe fever with thrombocytopenia syndrome virus
 - SARS CoV, MERS CoV

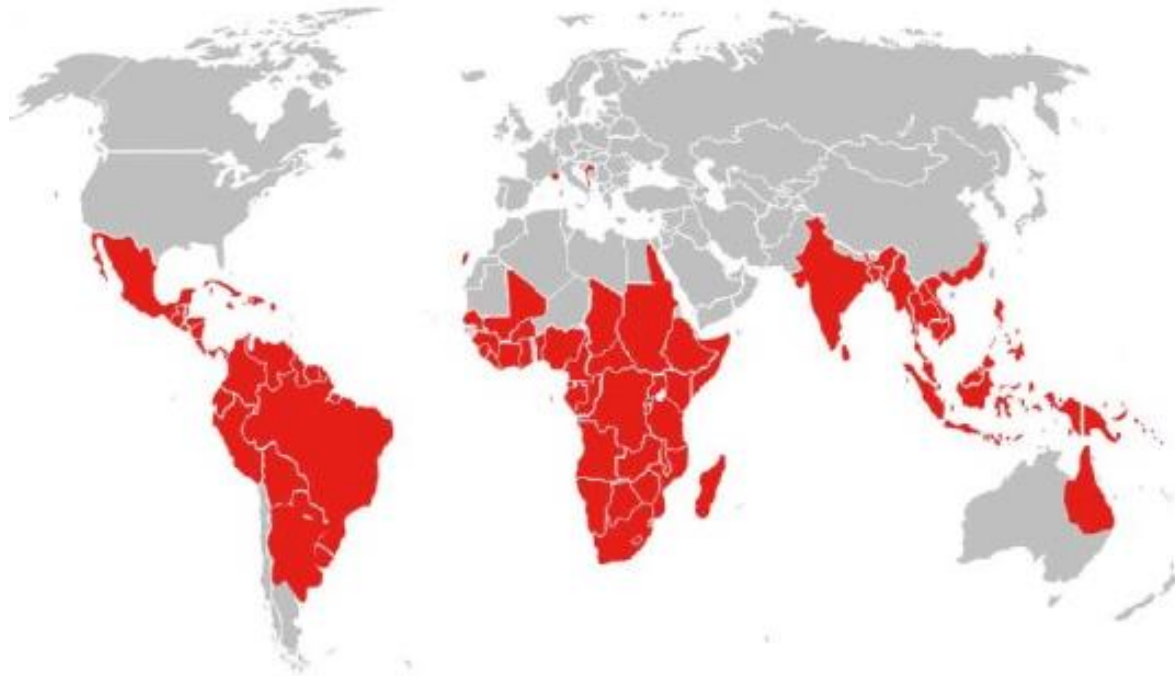
Dengue virus

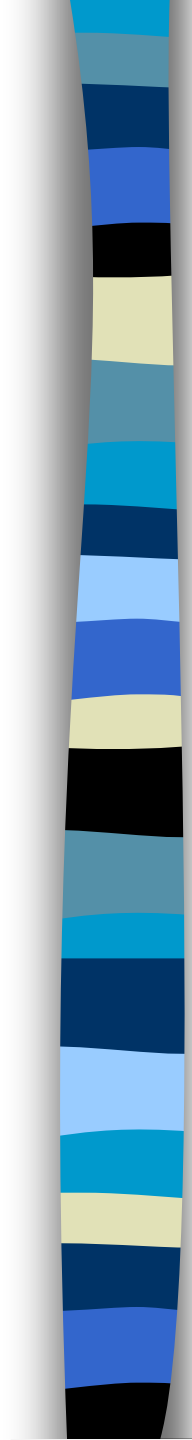
- Member of the *Flaviviridae*, *Flavivirus* genus
 - Mosquito borne, endemic in tropic and sub-tropical areas
 - 4 serotypes, possible 5th
 - All types cause haemorrhagic disease, shock
 - Previous exposure modulates disease on re-infection
- No vaccine, vector control



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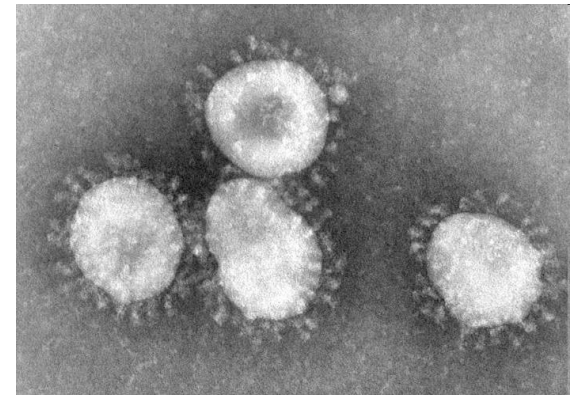
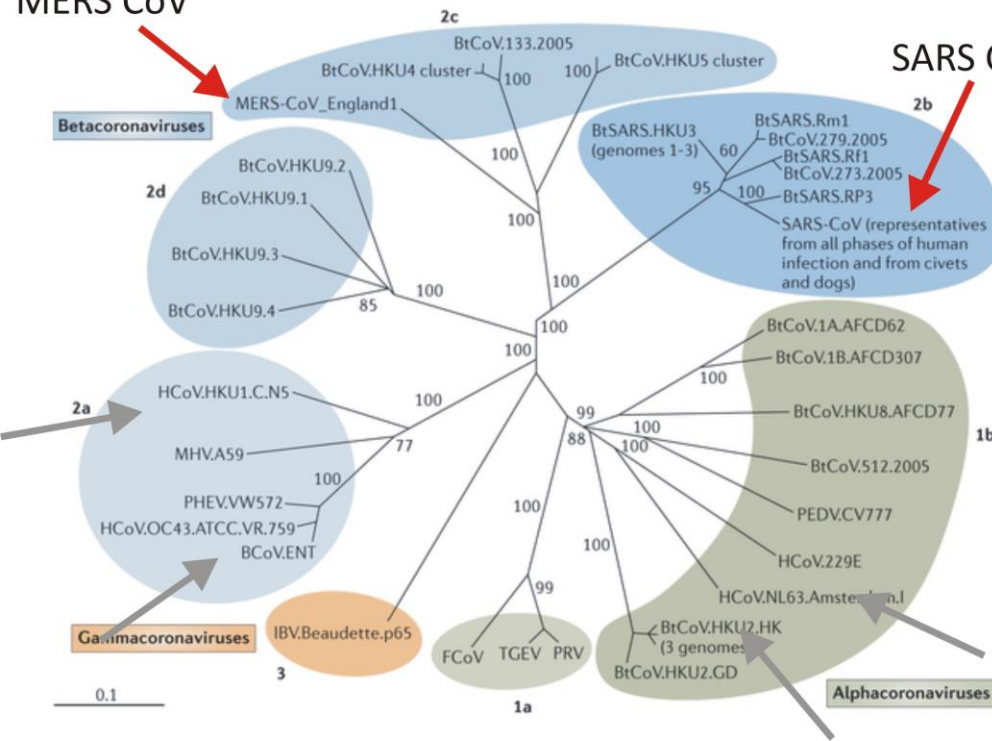
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- Blood donation risk
 - Viraemic phase on acute infection - two thirds of infections may be clinically inapparent
 - 0.03 – 0.31% viraemia frequency in Puerto Rico blood donors in outbreak years
 - Three transmission episodes in Hong Kong, Singapore and Puerto Rico, 5 infected recipients identified
 - No current routine screening or other interventions, amenable to NAT.

MERS Coronavirus

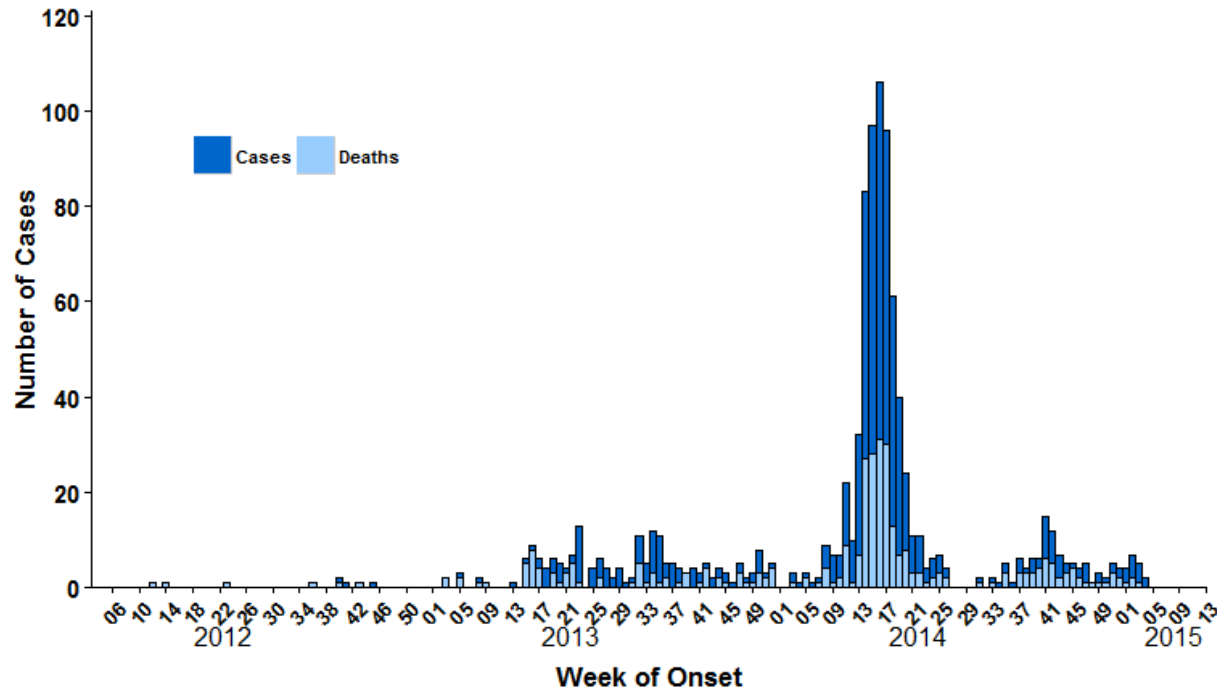
- Newly discovered member of the *Coronaviridae*
 - Suspected endemic reservoir in bats in the Middle East
 - Camels are an intermediate host for human infection
 - Typically occurs in those with close camel contact – some evidence for human-human transmission
 - Causes LRTI and multi-organ failure although typically in those with co-morbidities

MERS CoV



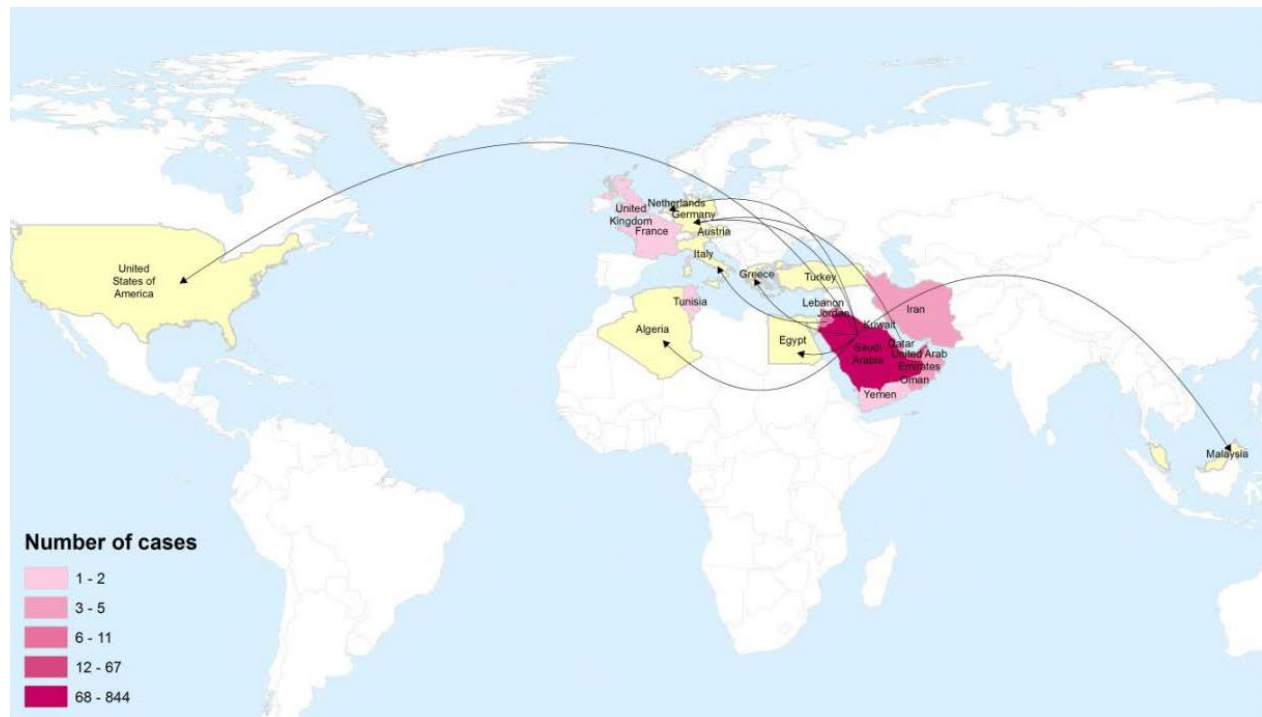
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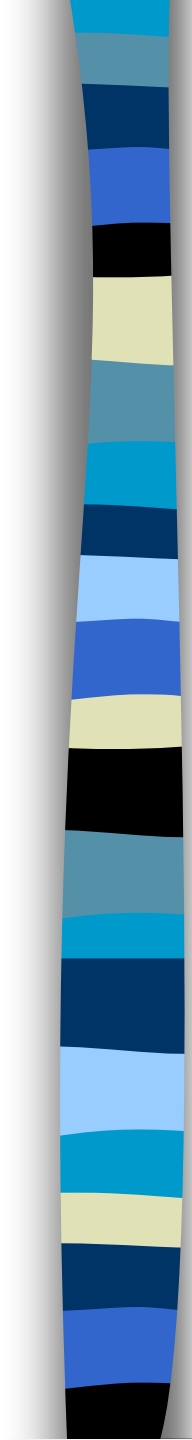
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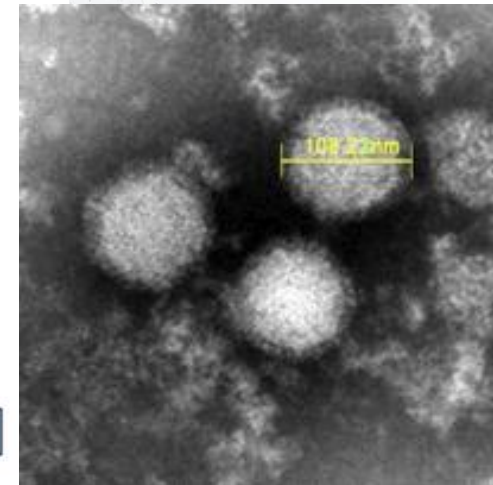
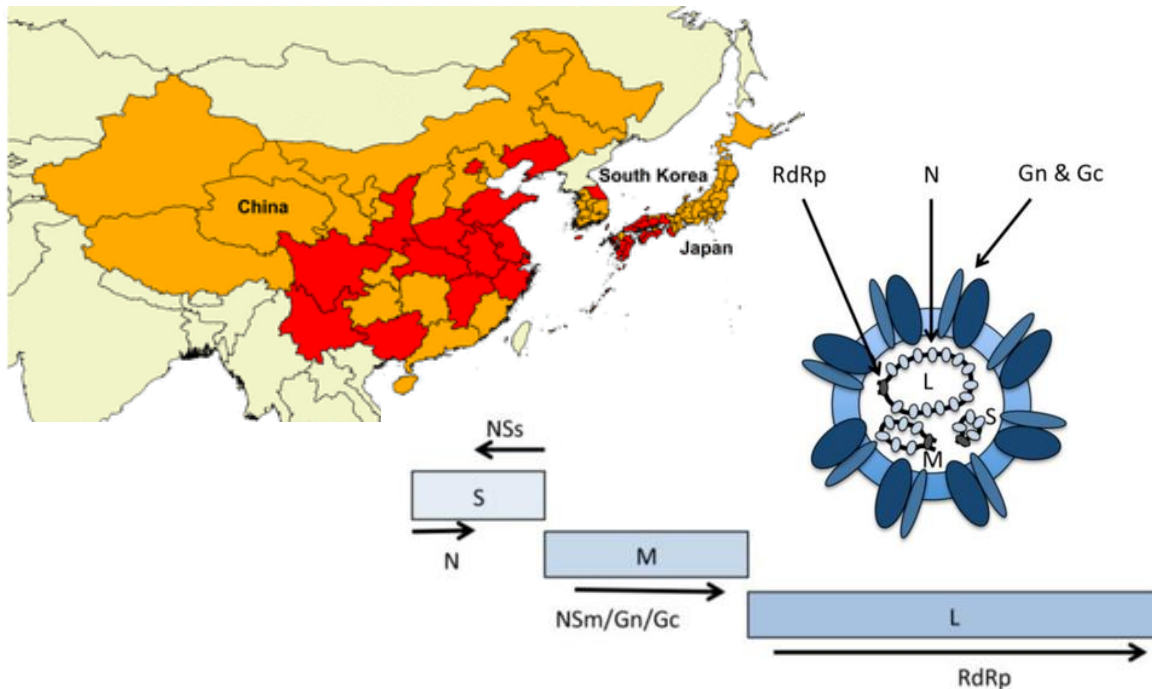


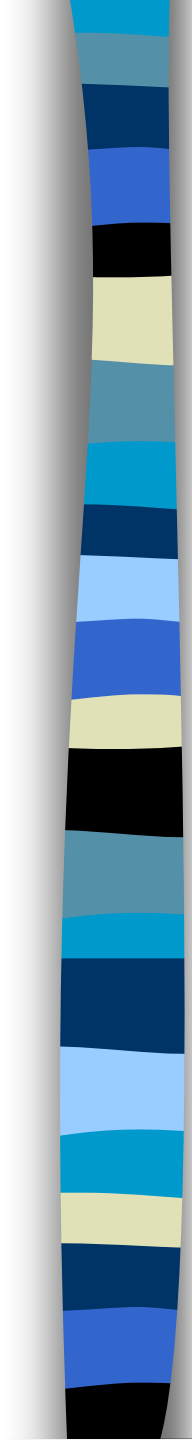
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 - Typically occurs in those with close camel contact – some evidence for human-human transmission
 - Causes LRTI and multi-organ failure although typically in those with co-morbidities
- Blood donation risk
 - Increasing incidence throughout Middle East
 - Nov, 2013: 160 cases, 68 deaths,
 - Mar, 2015: 1082 cases, 439 deaths,
 - Viraemic phase on acute infection, dynamics of virus persistence unclear
 - Low frequency of exposure in Saudi population reported*:
 - 10009 donors, >15 years of age
 - 15 seropositives identified, mainly male
 - Infectivity of seropositive individuals unknown

Severe Fever with Thrombocytopenia Syndrome virus (SFTSV)

- *Bunyaviridae*, genus *Phebovirus*
 - SFTS described in 2009
 - Causative virus discovered 2011 (Yu et al., Xu et al., 2011)
 - Epidemic in East Asia, tick borne (mainly *H. longicornis*)
 - High fever, thrombo/leucocytopenia
 - Multi-organ disease, immunopathology (cytokine storm)
 - High case/fatality rate (6%-30%)





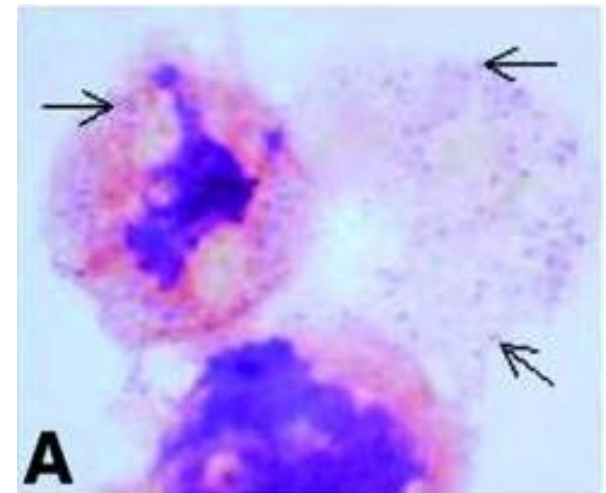
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- Blood donation risk
 - 71 from 986 seropositive (7.2%) in Zhejiang, China
 - 5.5% in SE China, higher in those with animal contact
 - Recently evaluated in large scale screen of Chinese blood donors*
 - 86 seropositive donations from 17,208 screened (0.5%)
 - All NAT-negative - ? Past infection
 - Two viraemic donations identified, low viral loads
 - Low estimated risk to blood safety.

*Zeng et al, *Transfusion*. (2015)

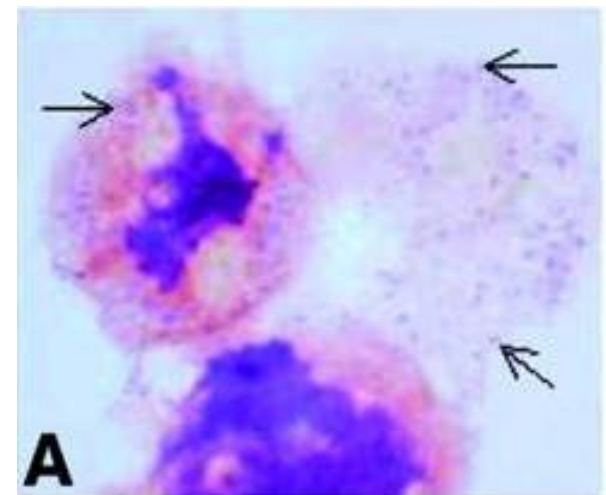
What else infects blood donors?

- Origin - Bradford, UK
 - Contamination found in hot water tank by amoebae (1991)
 - Cytoplasm full of small gram-positive organisms
 - Bacteria non-isolatable



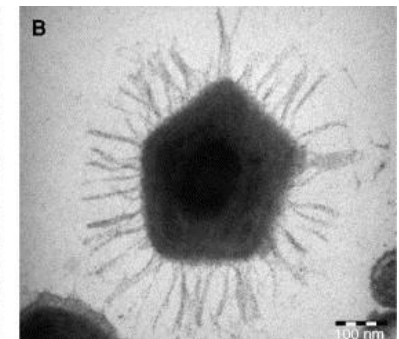
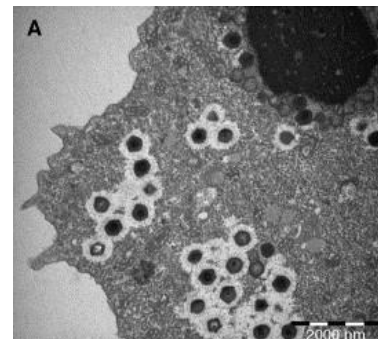
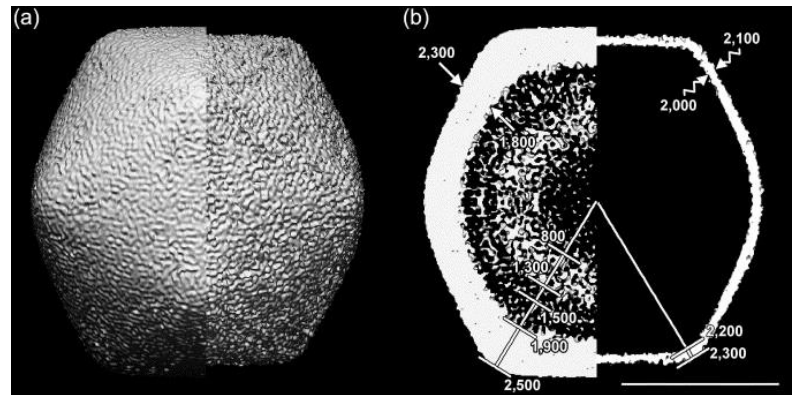
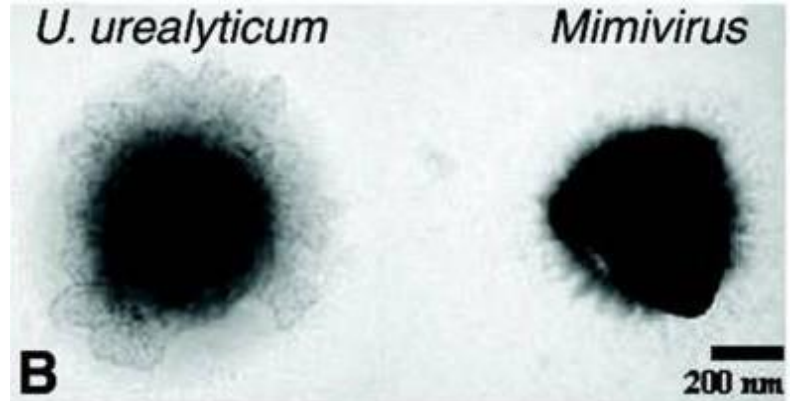
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 - Contamination found in hot water tank by amoebae (1991)
 - Cytoplasm full of small gram-positive organisms
 - Bacteria non-isolatable
- Analysed by bacterial taxonomists in Marseilles
 - Large genome (>1,000,000 bps)
 - No ribosomal RNA genes detectable
 - Unable to translate proteins



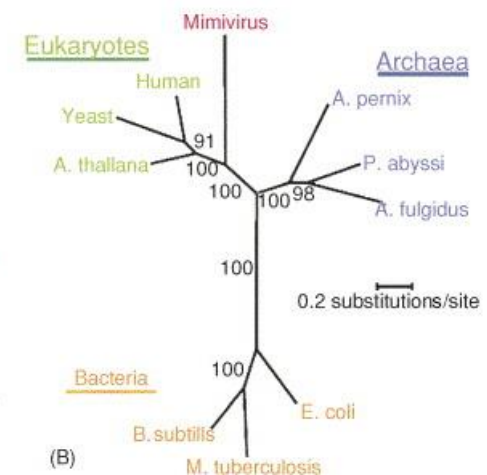
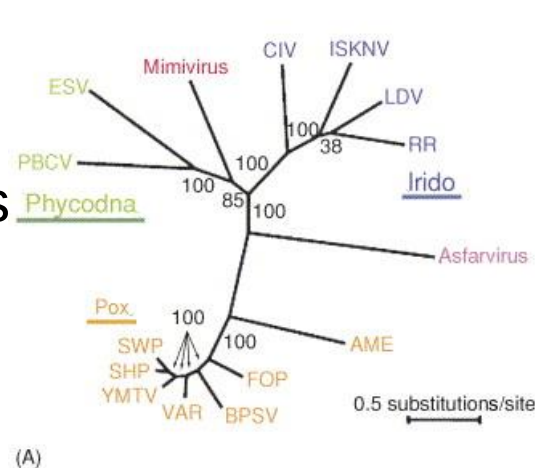
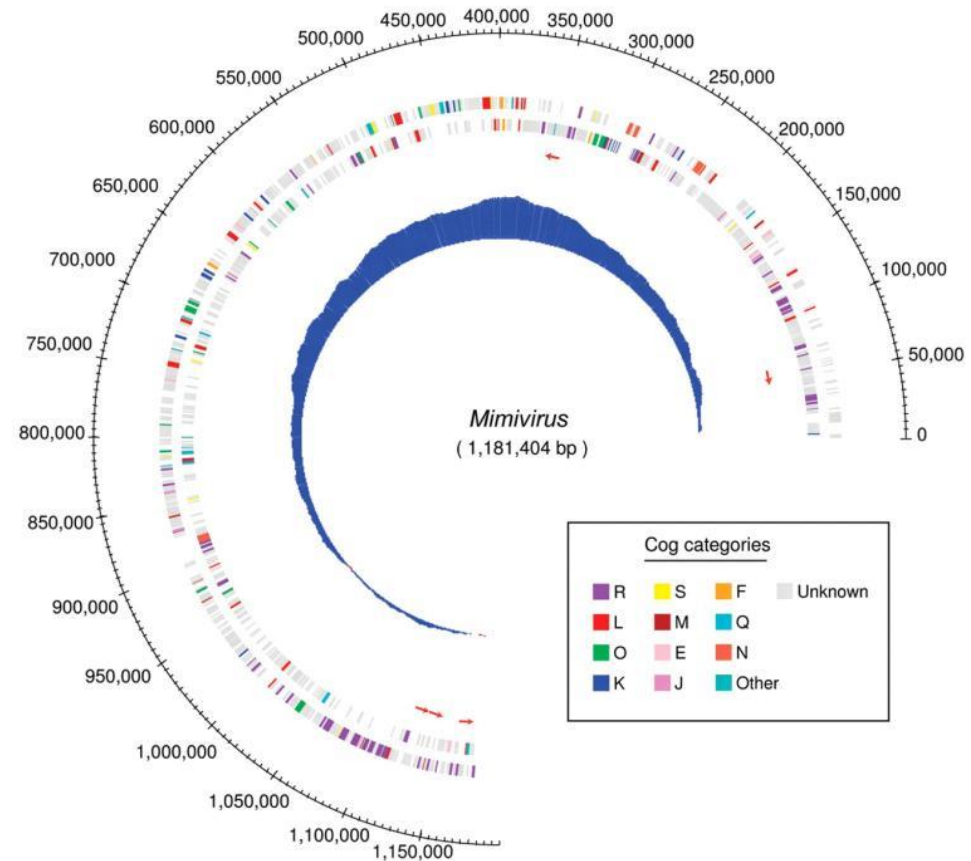
Mimiviruses

- Matches size of parasitic bacteria (*Mycoplasma*, *Ureaplasma*)
- Obvious shape differences from intracellular bacteria
- Closer EM investigation revealed enormous viral particles
 - Icosahedral symmetry
 - Complex multilayered capsid by cryo-EM
- Virus factories within cells
 - Cytoplasmic
 - Resemble replication complexes of smallpox



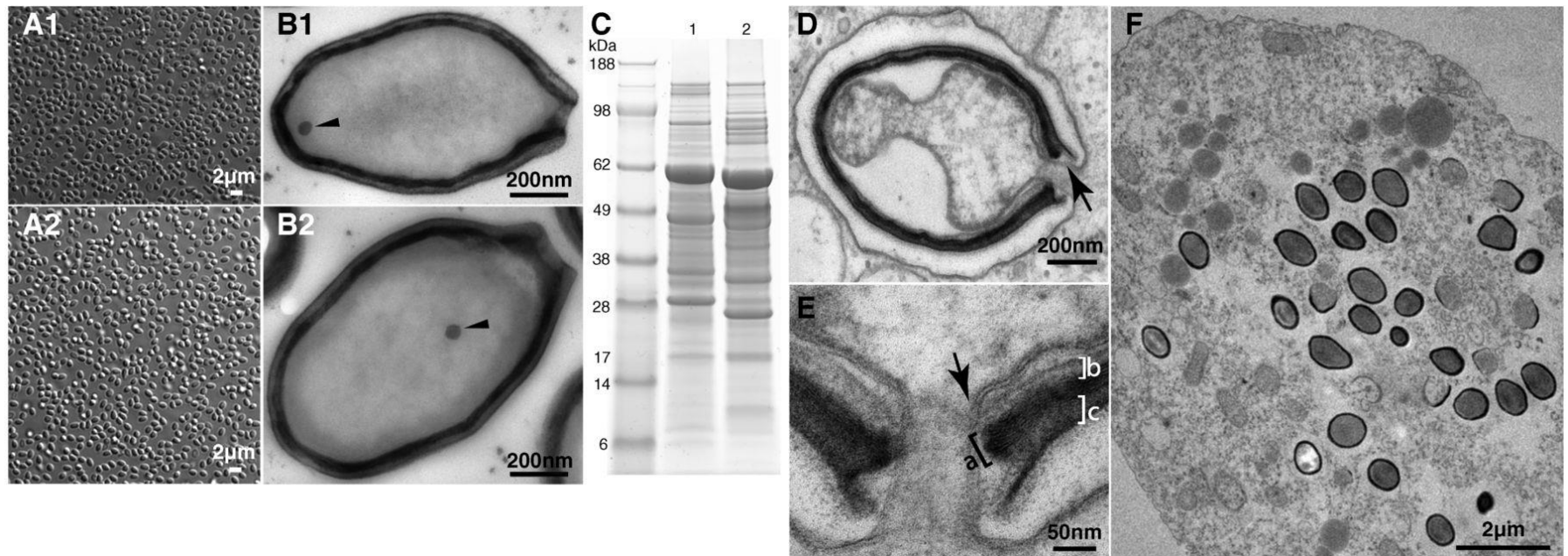
Mimiviruses

- Complete genome sequence:
 - dsDNA, 1200 kBp
 - Larger than mycoplasma (580 kbp) or ureaplasma (750 kBp)
 - No translation capability
- Linear DNA genome
 - Covalently closed ends
- Few translation-associated genes
- Contains some core genes found in all cellular organisms
- Virus structural genes similar to those of other larger DNA viruses



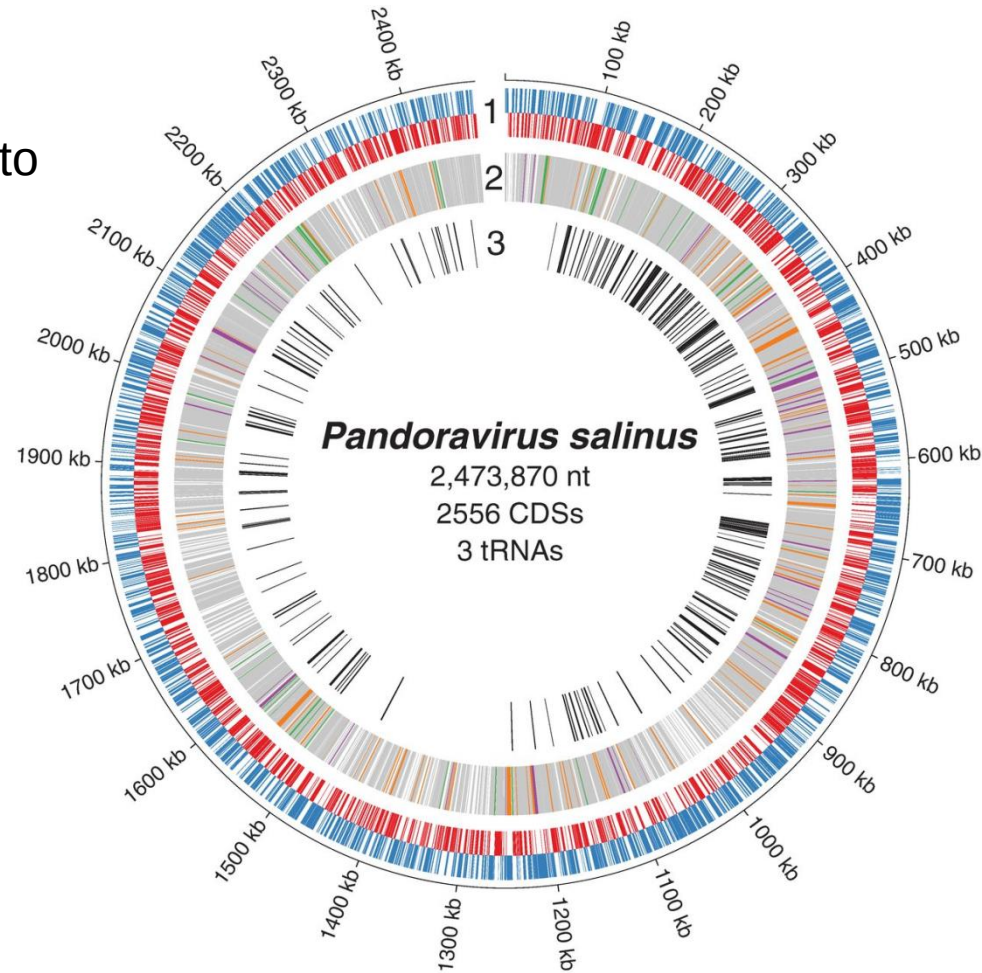
....and even bigger - Pandoraviruses

- Detected in amoebae in mud
 - *Pandoravirus salinus* - marine sediment in Chile
 - *Pandoravirus dulcis* - a freshwater pond in Melbourne
- Entry by endocytosis, fusion of internal membrane with ER, release of viral contents into the cell
- Breakdown of the nucleus, appearance of assembling virus particles
- Cell lysis and release from cells



The *Pandoravirus salinus* genome

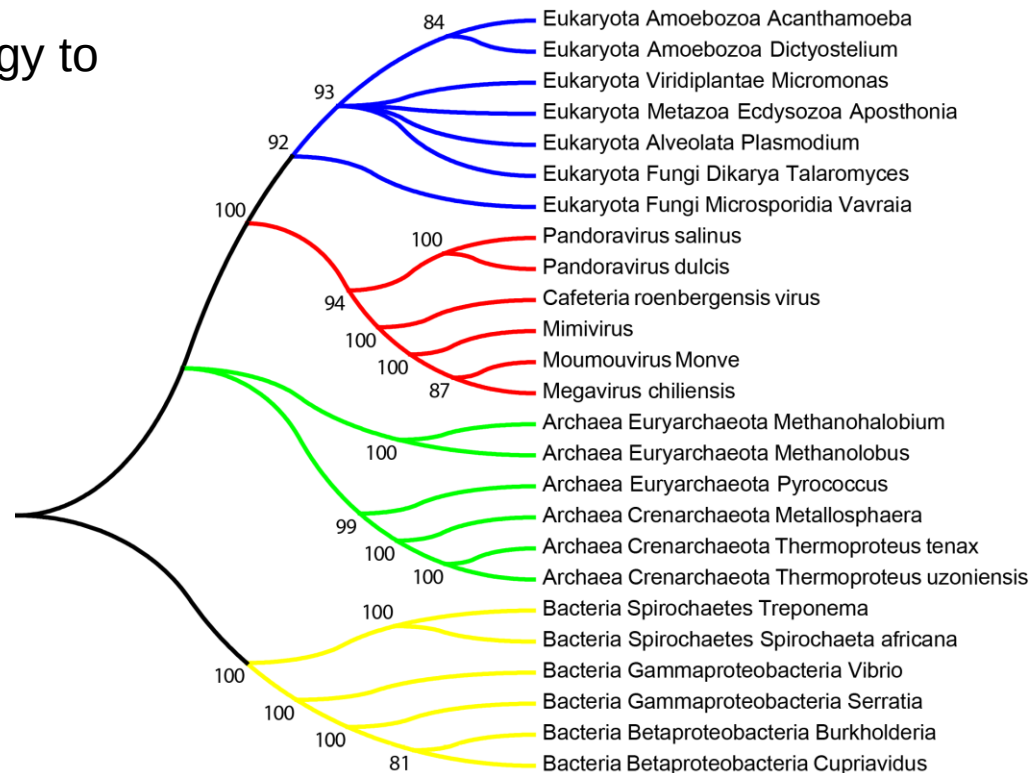
- Similar size and gene count to most bacteria
- Predicted to encode 2556 proteins
 - 2370 show no homology to known proteins
 - 17 distantly related to mimivirus genes
 - Other matches to:
 - Eukaryote (n=101)
 - Prokaryote (n=43)
 - Other virus (n=42)
 - Host derived (n=92)
- No ribosomal RNA or protein sequences
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The *Pandoravirus salinus* genome

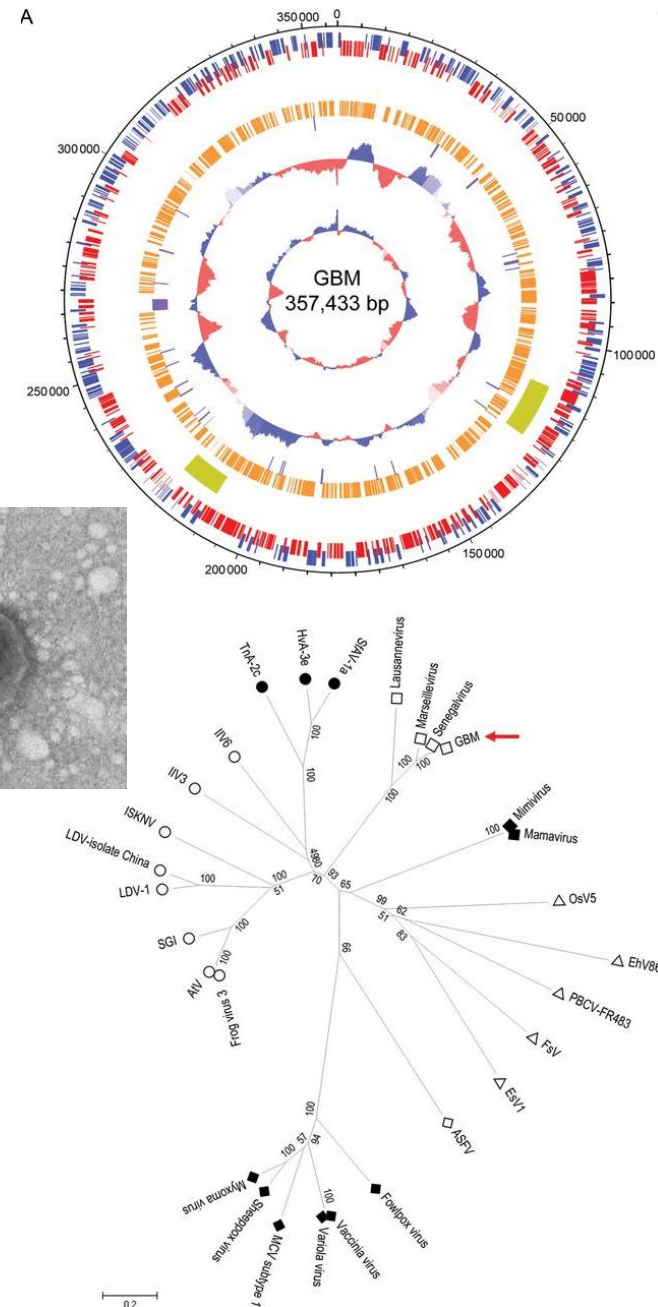
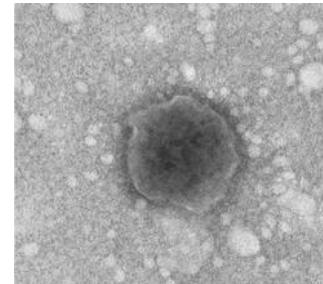
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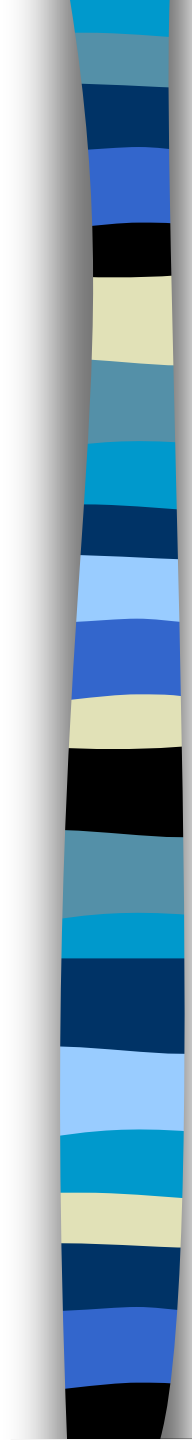
DNA polymerase tree



Giant Blood Marseillevirus

- New member of the *Megavirales*
 - 357,000 base pairs
 - Remarkably frequent infection in the general (donor) population:
 - 4% of 172 donors viraemic
 - 13% seropositivity
- Potentially transmitted by blood transfusion
 - Higher rates of viraemia (9%) and seropositivity (23%) in thalassaemia patients
- Infections appear non-pathogenic and persistent
 - Megavirales typically infect lower eukaryotes
 - Findings require wider confirmation





Virus discovery challenges

■ Technology

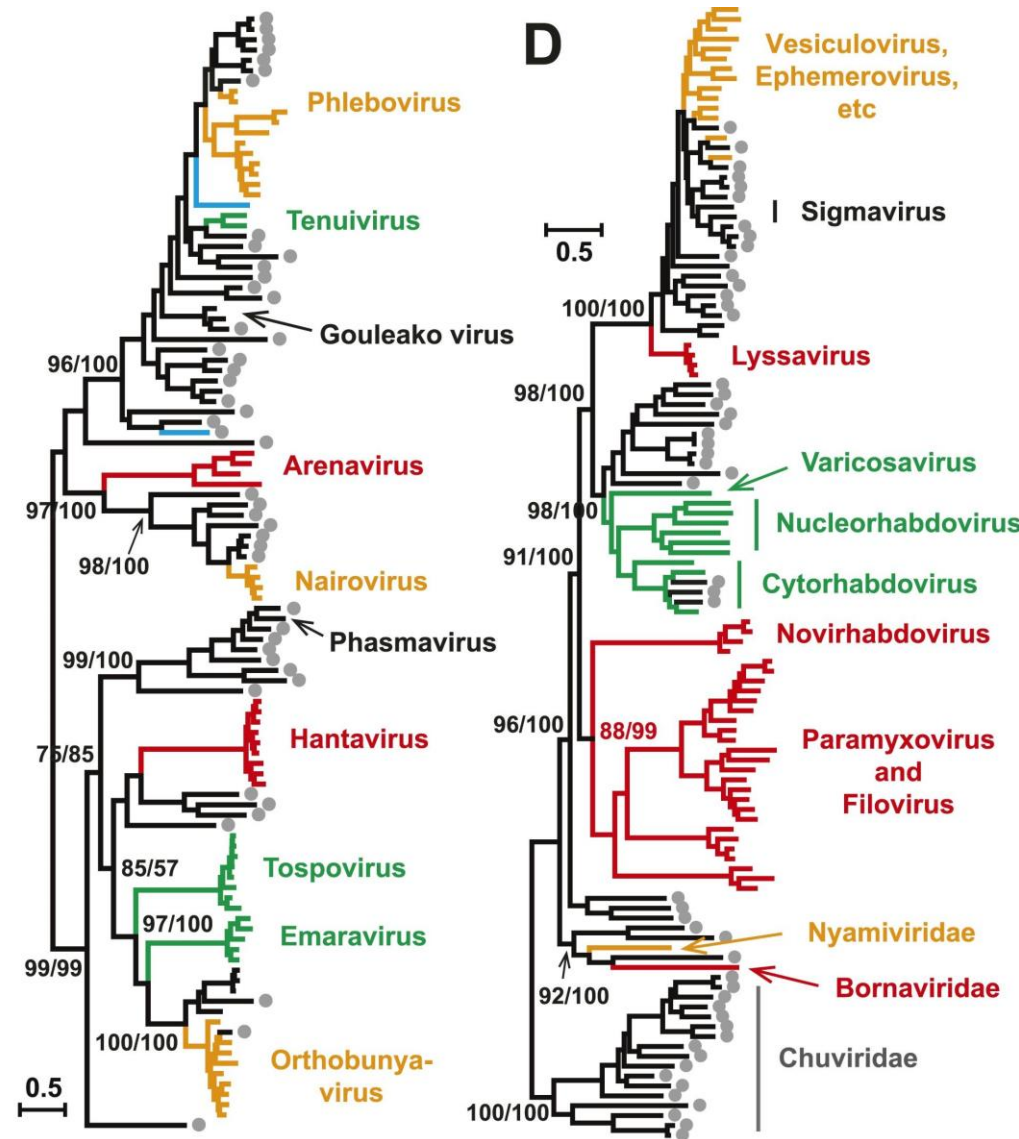
- Large proportion of reads are unidentifiable, irrespective of source
- Newly discovered viruses almost invariably only identified by matches to an existing virus family or group
- Few newly discovered viruses fall outside existing taxons
- NGS lacks an ability to find anything genuinely new

■ Explaining clinical / veterinary disease

- Are there major unexplained diseases with a suspected but unidentified viral cause? (*ie.* like AIDS/HIV & NANBH/HCV)
 - Next few years crucial in resolving this
 - Rate of post-transfusion disease low
- No negative predictive value
 - Negative studies remain unpublished
- Have existing virus detection methods been more effective than thought?

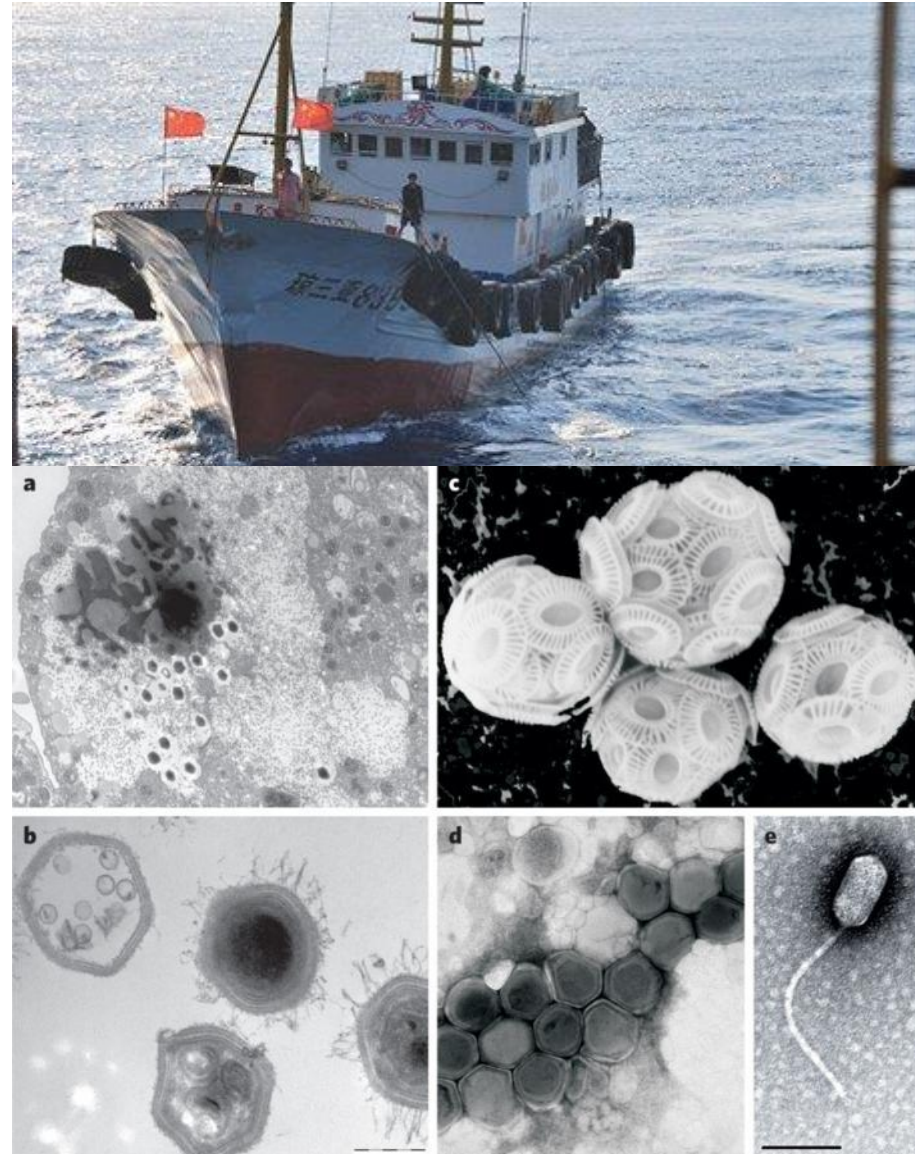
Arthropods

- Take 8 sacks of insects
- Apply NGS
- Identify all RNA polymerase sequences
 - Conserved enzymatic motifs
- Add to the existing set of sequences of minus-strand viruses
 - Large degree of fill-in of existing taxa
 - Taxon assignments problematic
 - Scope to be misleading



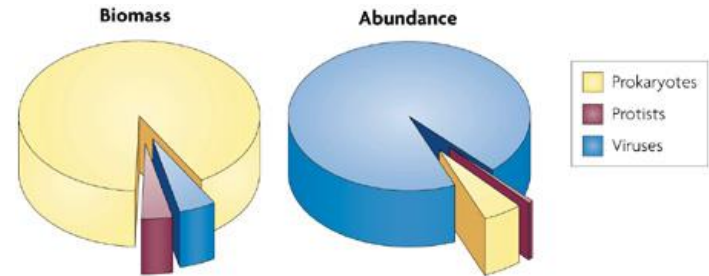
Wider surveillance – the oceans

- The sea is the largest microbial environment
- Contains most of the world's biomass
 - Bacteria, archaea
 - Minority contribution of eukaryotes
- Surveillance for microbial diversity
 - Surface and deepwater sampling
 - TARA Ocean project, 3 year circumnavigation of main oceanic zones
 - Multiple depth sampling
- Mass sequencing of extracted DNA/RNA
 - Most sequences obtained unidentifiable

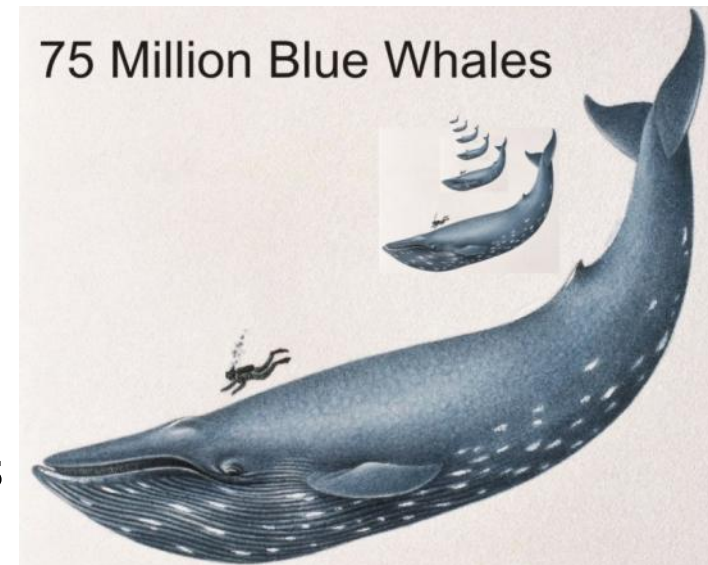


Ocean surveillance

- Viruses are the most abundant life form
 - 10^{30} virus particles in the sea
 - 5% of biomass
 - Major environmental role
 - Kill 10% of ocean's bacteria and archaea every day
 - Likely determining role in microbial abundance
- Enormous genetic diversity
 - DNA phages (50%)
 - Mimivirus-like viruses (10%)
 - Most unidentifiable sequences likely correspond to novel gene sets and functions in viruses
- May be organisms outside conventional cellular and viral domains

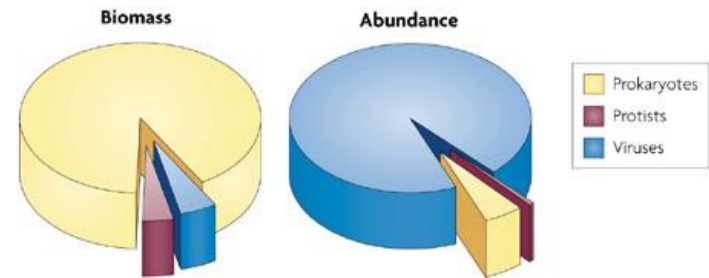


Nature Reviews | Microbiology

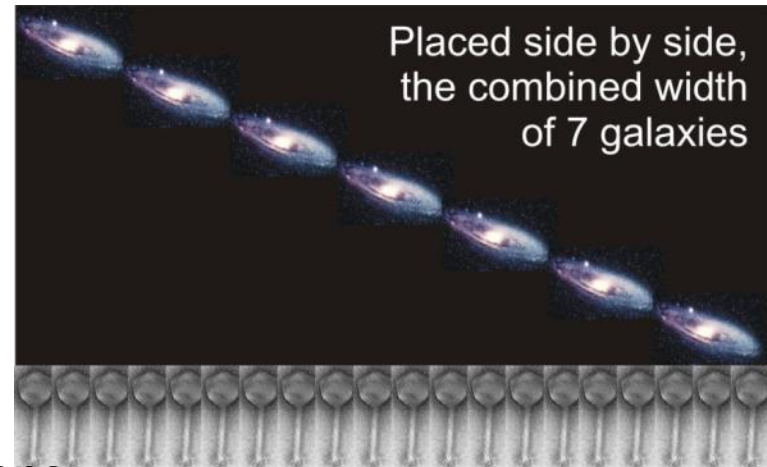


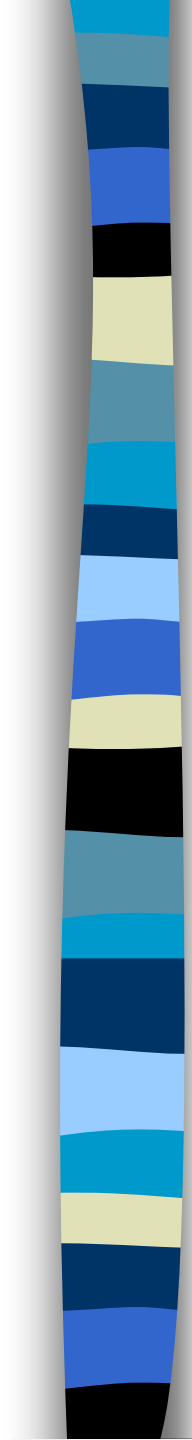
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Conclusions

■ Viral diversity

- Pervasive presence of viruses in the environment
- Vast ecosystem of virus / host (eu- and archaeo-bacteria, protists) interactions to explore
- By contrast, humans seem to possess a remarkably sterile internal environment
- A case would have to be made for a major role of new viruses in existing human disease

■ The future

- Improvements in bioinformatics and technology needed
- Methods for longer accurate reads needed in place of the current assembly of short fragments (eg. Illumina)
- A better understanding of deep phylogeny and relationship with virus classification