Principles of clinical virology

Structure, Pathogenesis, and Uses of New Diagnostics

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Epidemics

- Plague of Athens (436 B.C.) described a distemper-like epidemic with high mortality – almost certainly due to measles
- Epidemics - Rome, China AD165, AD251
- Morbilliform rash and consequences considered normal process of development
- Ongoing outbreaks in NSW 2010-2016

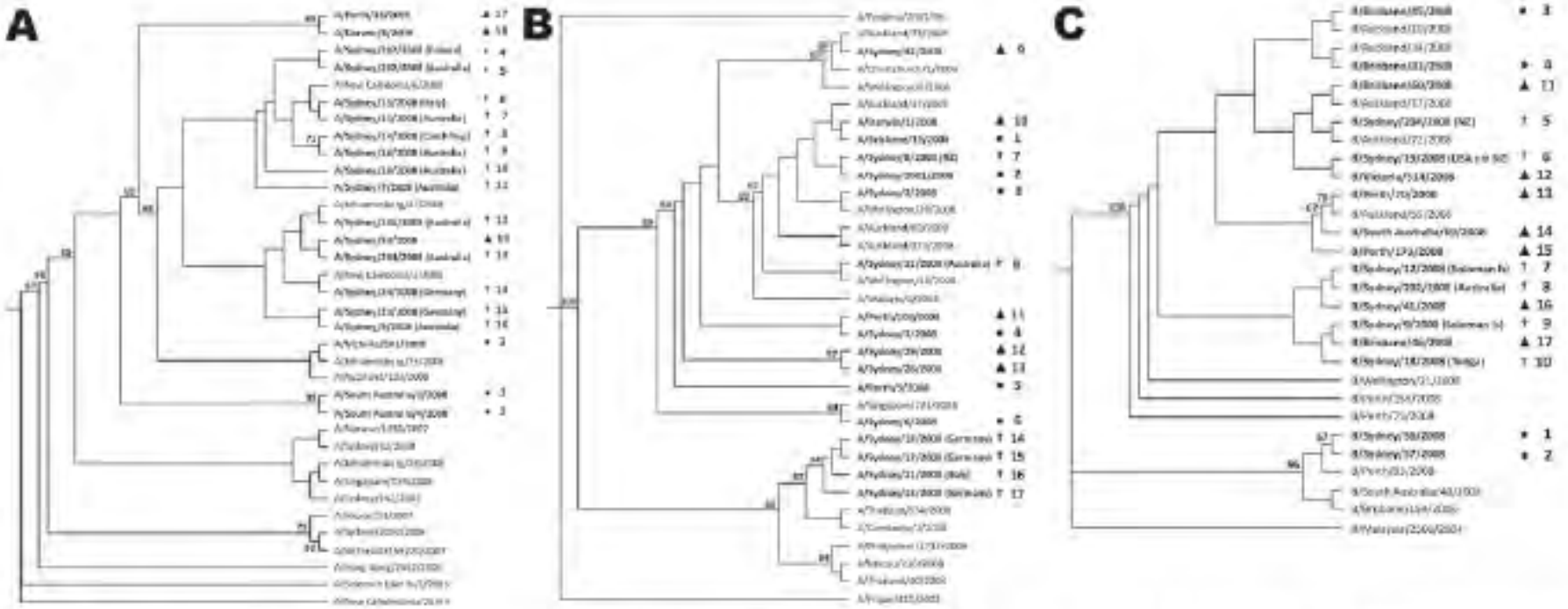

Figure 3. Phylogenetic trees illustrating relatedness of hemagglutinin sequences from influenza A (H1N1) (A), A (H3N2) (B), and B/Malaysia-like viruses (C) from pre–World Youth Day 2008 (WYD2008) Australian isolates (●), WYD2008 isolates (†), post-WYD2008 Australian isolates (▲), and related international isolates. Trees were constructed by using maximum-likelihood (DNAml) in PHYLIP. Only bootstrap values ≥60 are included.

Influenza Outbreaks during World Youth Day 2008 Mass Gathering

Christopher C. Blyth,[1] Hong Foo, Sebastiaan J. van Hal, Aeron C. Hurt, Ian G. Barr, Kenneth McPhie, Paul K. Armstrong, William D. Rawlinson, Vicky Sheppeard, Stephen Conaty, Michael Staff, and Dominic E. Dwyer, on behalf of the World Youth Day 2008 Influenza Study Group[2]

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 16, No. 5, May 2010

"With 7.4 billion people, 20 billion chickens and 400 million pigs now sharing the earth, we have created the ideal scenario for creating and spreading dangerous microbes."

[The real threat to national security, Michael Osterholm 2017]

OUTLINE

1. What is a virus
   - Characteristics
   - Structure
   - Replication
   - Definitions
2. How viruses cause disease
   - Molecular principles
   - Disease pathogenesis
   - New ways of examining virus pathogenesis
3. Clinical virology and diagnosis of viral illness
   - Principles
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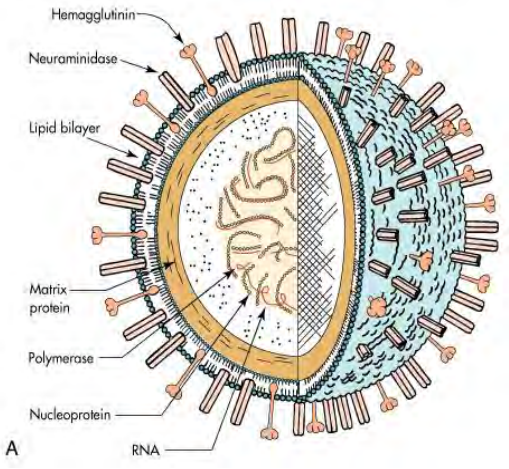
A virus is a molecular genetic parasite that uses cellular systems for its own replication

Filtrable agents

Delivery system surrounding a payload



ICTV Definition

- “a virus species is a polythetic class of viruses that constitute a replicating lineage and occupy a particular ecological niche”
- “polythetic class” members have several properties in common, although not necessarily all share a single common defining property

- That is, members of a virus species defined collectively by a consensus group of properties
- Virus species differ from higher viral taxa, which are “universal” classes and as such are defined by properties that are necessary for membership

Viruses

- Viruses are the simplest organisms, containing DNA or RNA, but not both
- RNA viruses are more diverse and replication often error prone

- Enveloped (environmentally unstable) and non-enveloped (environmentally stable)

Viruses have life

- Can be killed
- Can become extinct
- Undergo Darwinian selection
- Subject to evolutionary biology
- But
  - Have no sexual exchange process
  - Species is defined by its lineage
  - Species is a class that occupies a replicating lineage and occupies an ecological niche

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Viruses have life

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  - Species is a class that occupies a replicating lineage and occupies an ecological niche and can be defined by phylogenetic analysis

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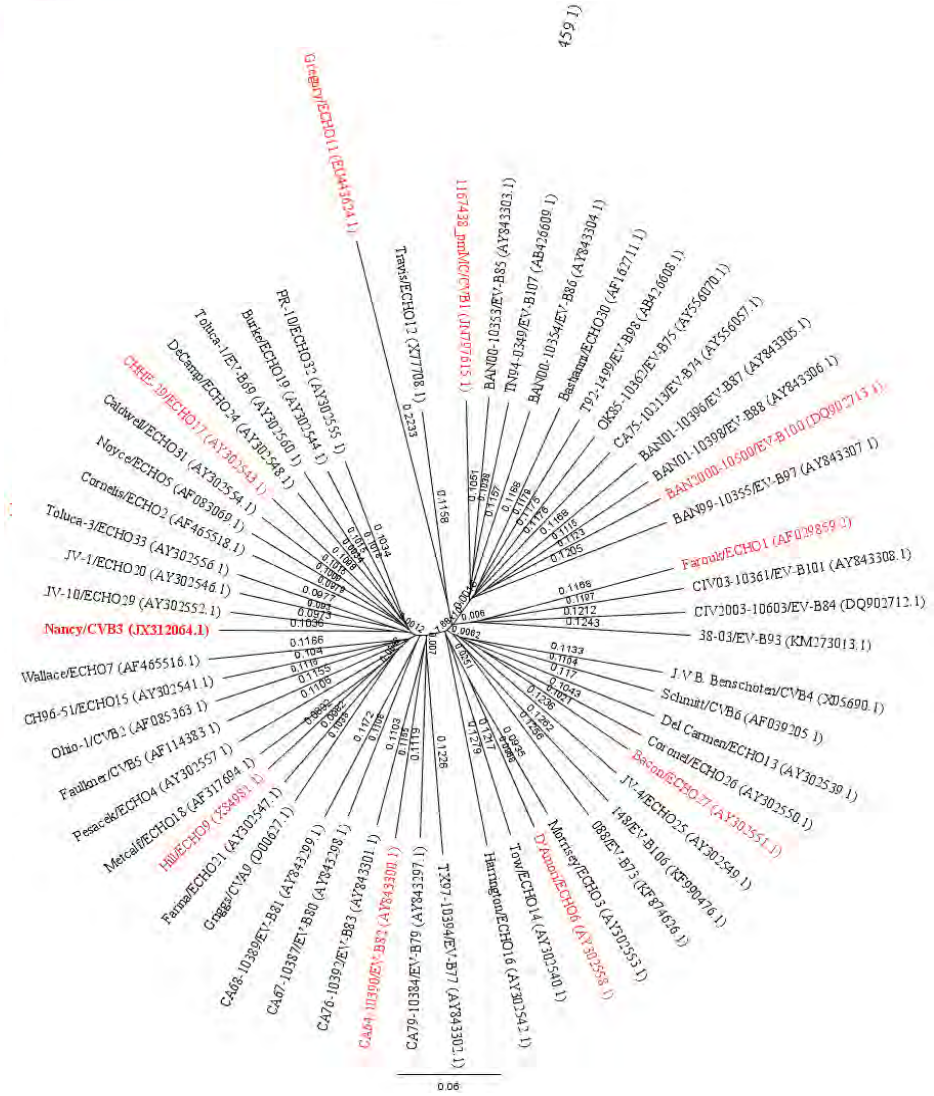
Classification and phylogeny

- Viral nucleic acid + virus capsid + envelope
- Other characteristics:
  - Genomic makeup e.g: Caliciviruses
  - Virion structure – EM appearance e.g: herpes
  - Replication strategy
  - Virion antigenicity e.g: adenoviruses, serological distinction MVE / JE / WNV
  - Virion chemical characteristics, stability
  - Diseases caused in the host e.g: hepatitis
- Phylogeny only uses nucleic acid or aa sequence

So what is phylogenetics and how does it help

- Phylogenetics or Phylogeny is study of evolution, diversity
  - How different organisms and species are related to each other
- Term *phylogeny* used in 1866 Haeckel, then Darwin
- Made of Greek *phylos* = race with *geneia* = origin



Steps in phylogenetic analysis

- Determine or obtain previous online sequences
- Align sequences so homologous nt (or aa) are in line with each other
- Perform calculations to make a tree, with branches determined by similarity of sequences
- Test trees for best fit of the data

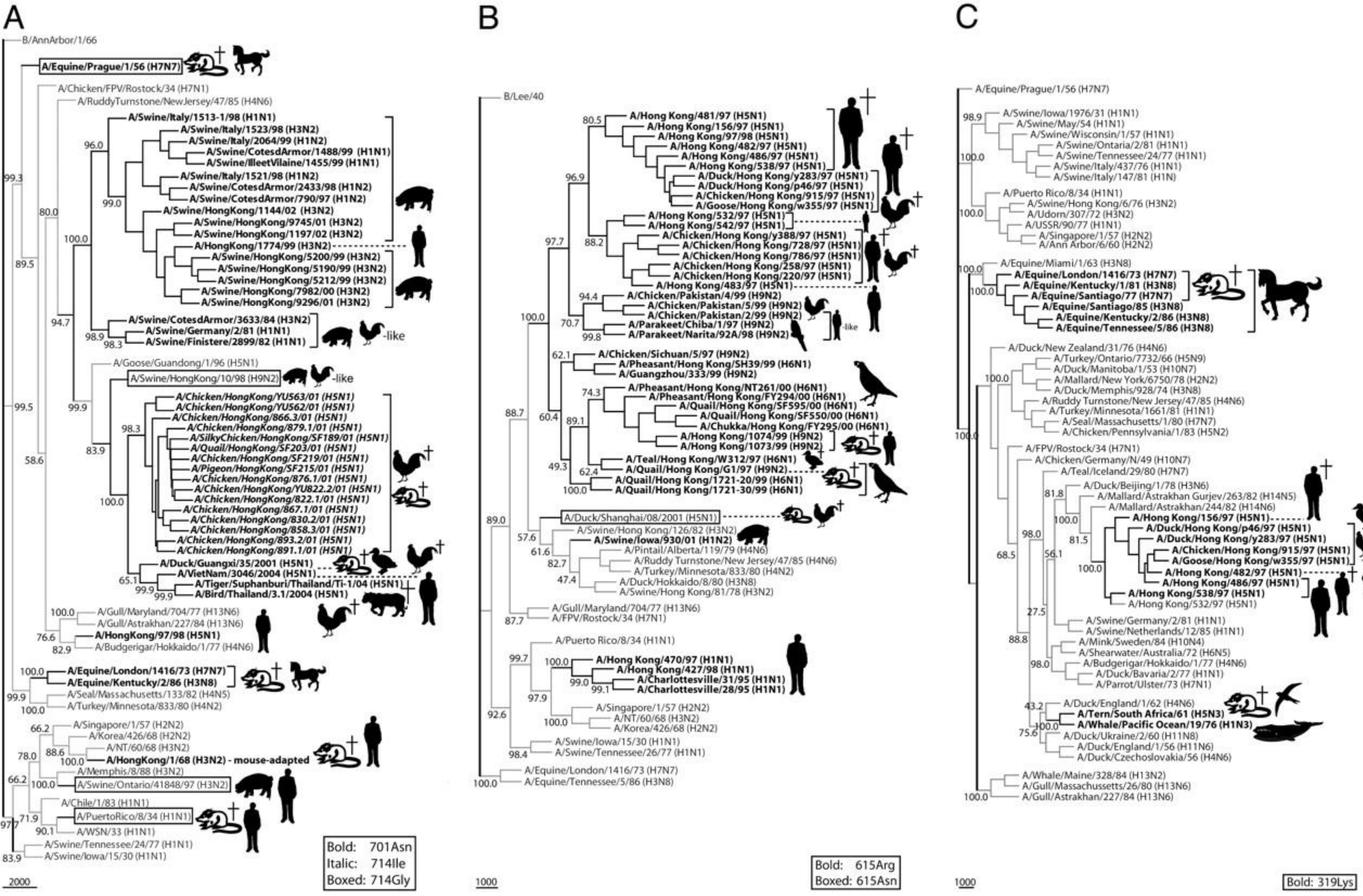
Alignments and trees

- Align
  - AAACTGAATG
  - AAACCGATTG
  - Differences real or sequence errors
- Construct trees based on computer-search for tree most consistent with the data set
  - Evolutionary distance
  - Maximum parsimony
  - Maximum likelihood

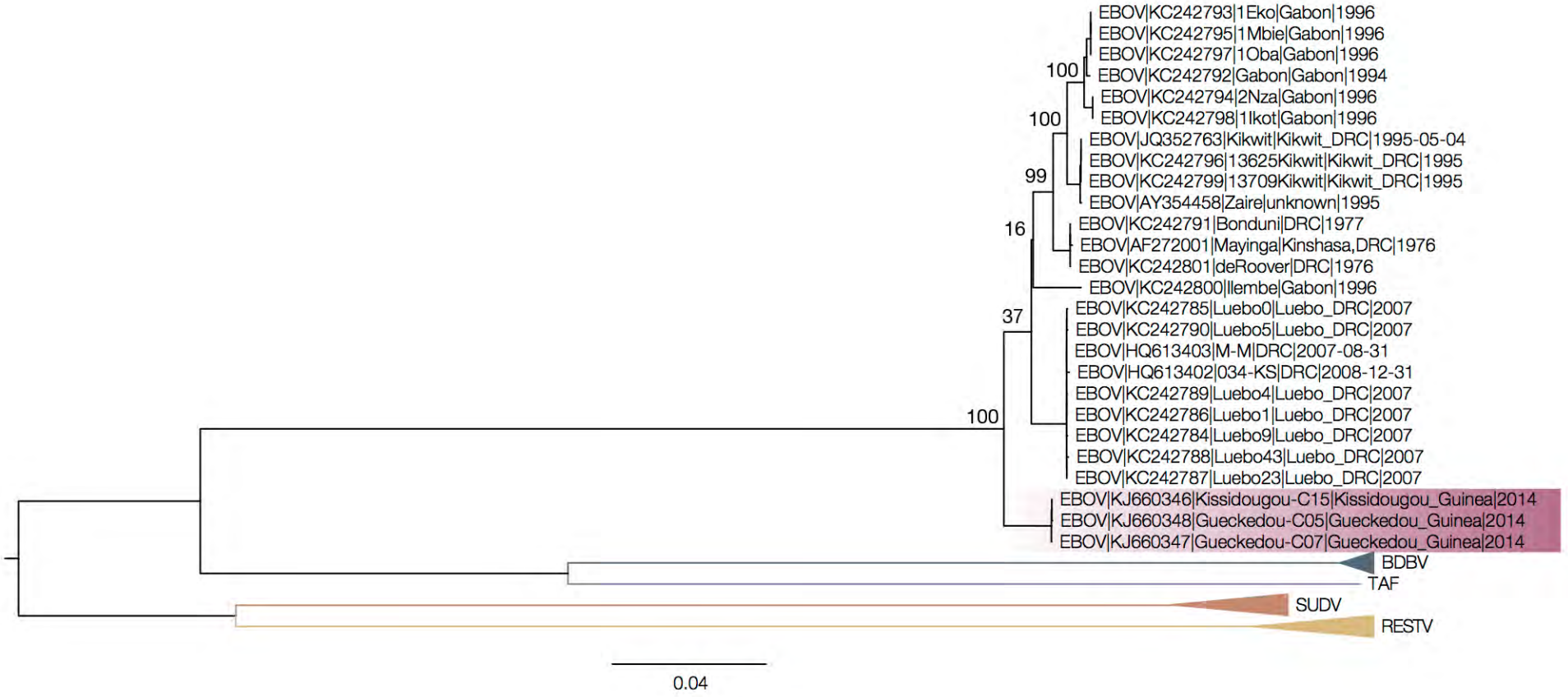
Things that may interfere

- Nucleotide biases (G+C)
- Rapidly evolving lineages
- Misalignment and slippages of sequence from multiple repeats, hompolymeric runs
- Taxon selection affecting outcomes – wrong outliers

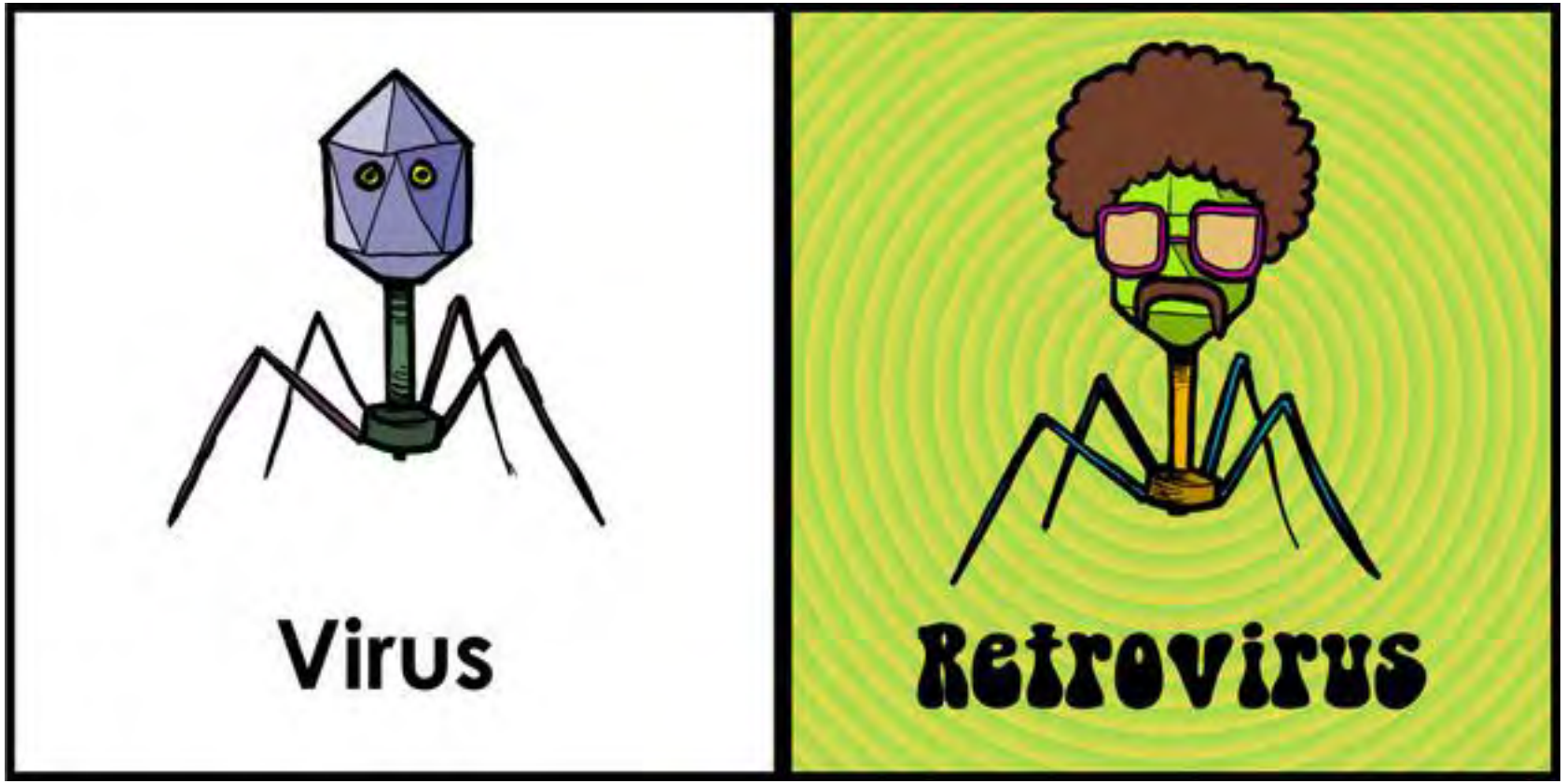
Phylogeny of influenzaviruses



EBOV Phylogeny- Guinea outbreak sequences are divergent lineage



[Dudas 2014]


Virus
Retrovirus

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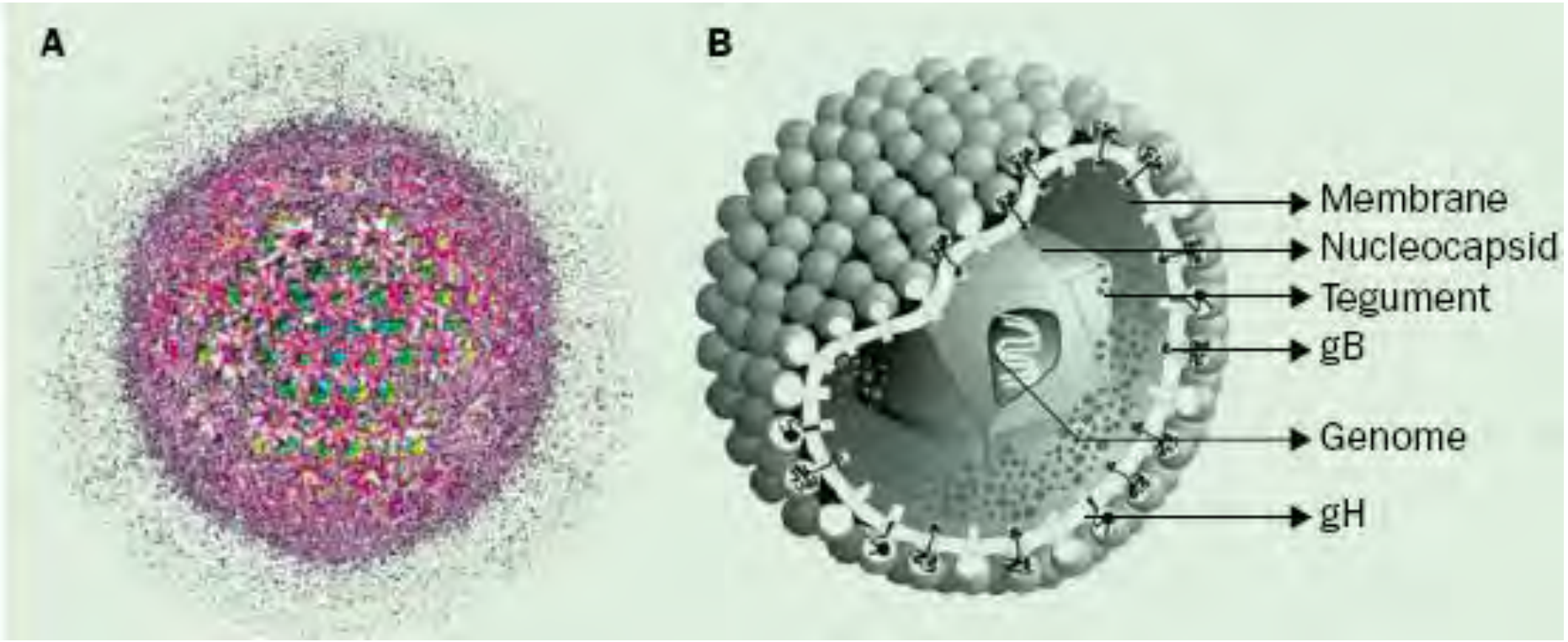
Structure

- Viruses are:
  - not cells
  - dependent upon the cell they infect. Inside cells they can replicate, outside cells they can be transmitted, but cannot replicate (grow)
  - sometimes viruses integrate their nucleic acid into the host cell genome



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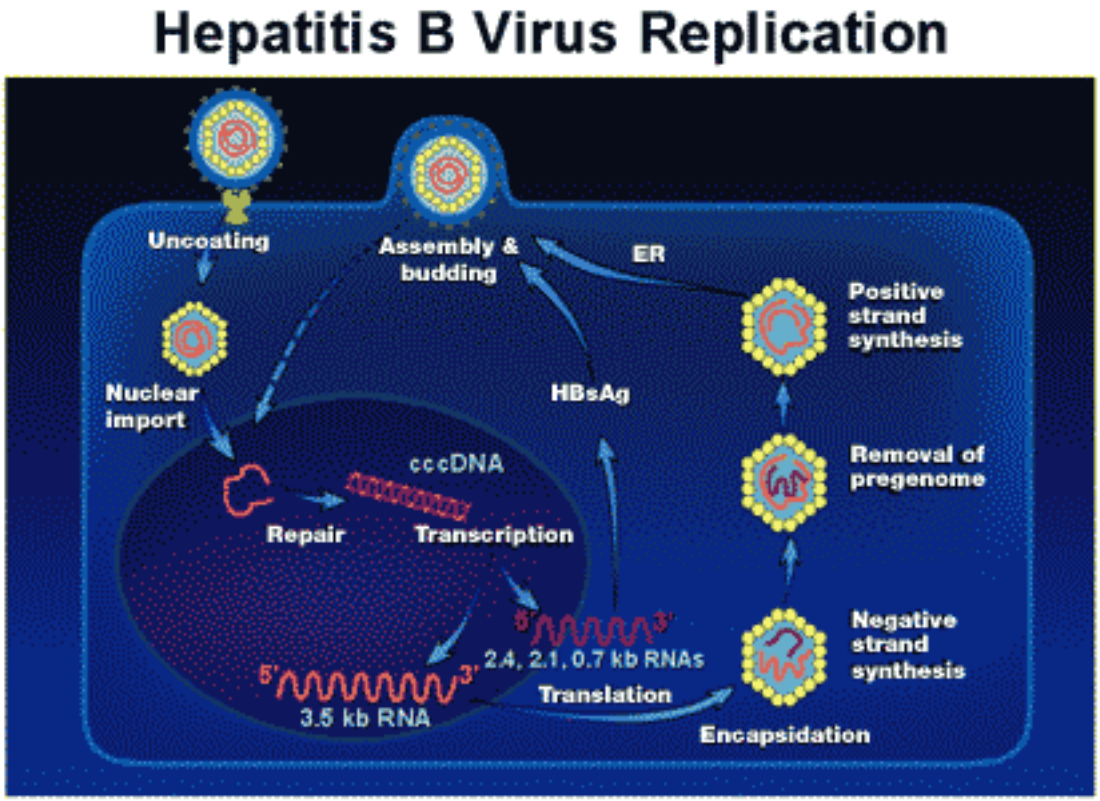
Virus replication
The delivery system and the payload



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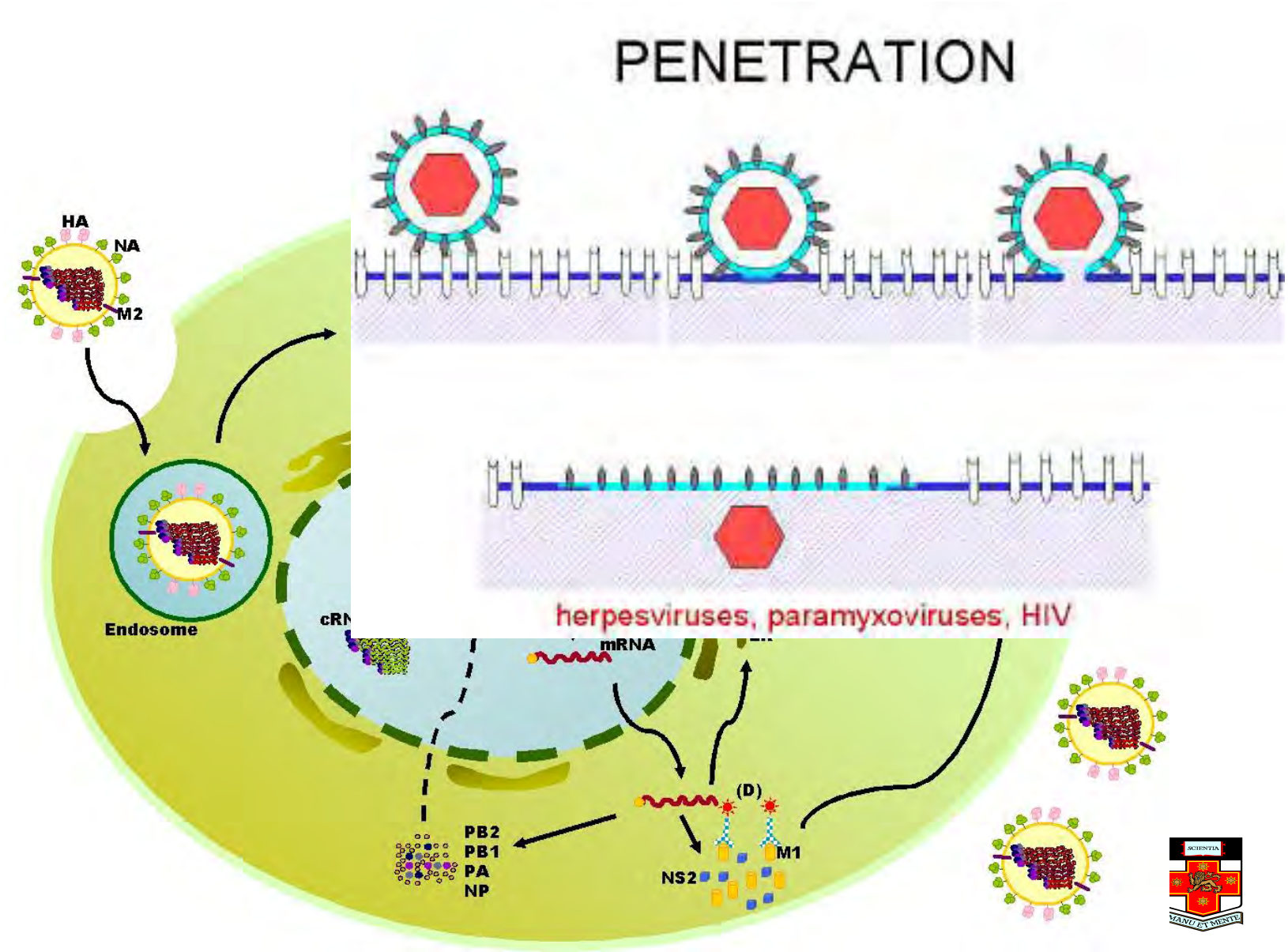


Virion Architecture
The delivery system

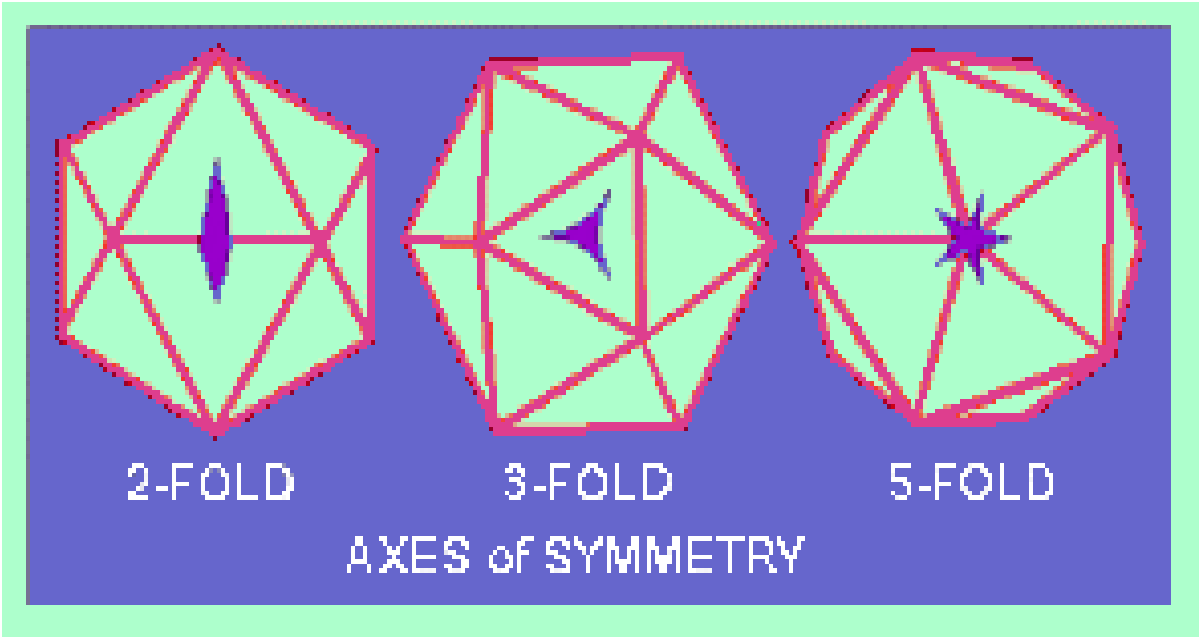
Architecture of virions regardless of host is based on two simple themes:

Sphere – normally in the form icosahedron (cubical)
- Best way of producing a shell of equivalently bonded identical structures
- Minimum free energy state
- Strong structure that can enclose a maximal volume

Helix – cylindrical shape (spiral staircase)

Virion Architecture – icosahedron

An ICOSAHEDRON is composed of 20 facets, each an equilateral triangle, and 12 vertices (corners)

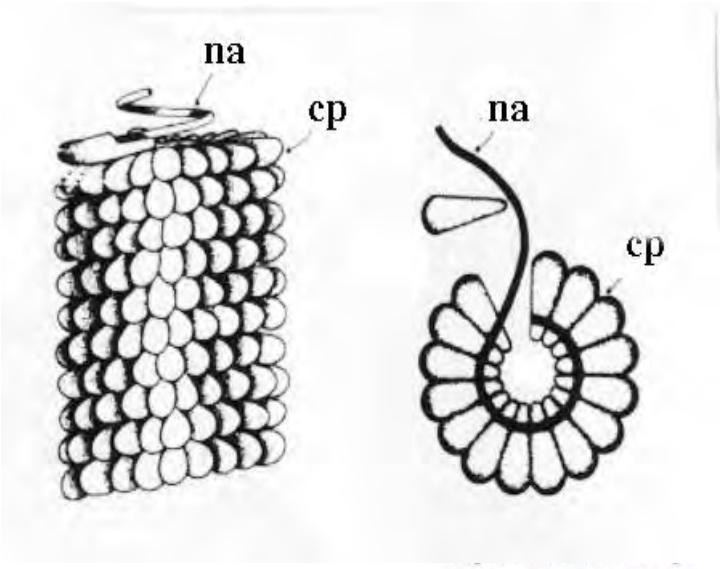


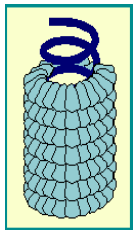
Helical viral structure

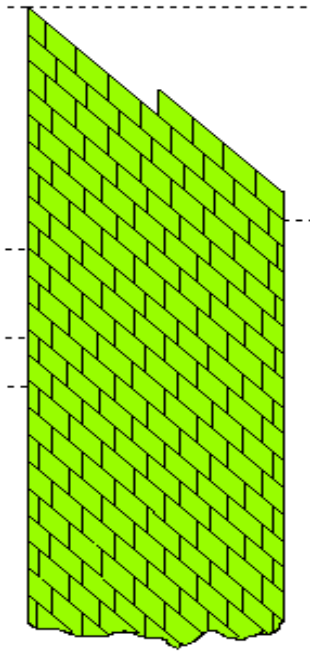
Several RNA viruses undergo self assembly as a cylindrical nucleocapsid. *(hollow tube)*

The viral RNA forms a spiral within the capsid structure

Each capsomer consists of a single protein







Classification

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Viruses as a molecule

- ssRNA – most diverse Noro, HCV, HIV
- dsDNA – Adeno, CMV, HSV, Variola
- dsRNA – Rota
- ssDNA – least diverse PVB19

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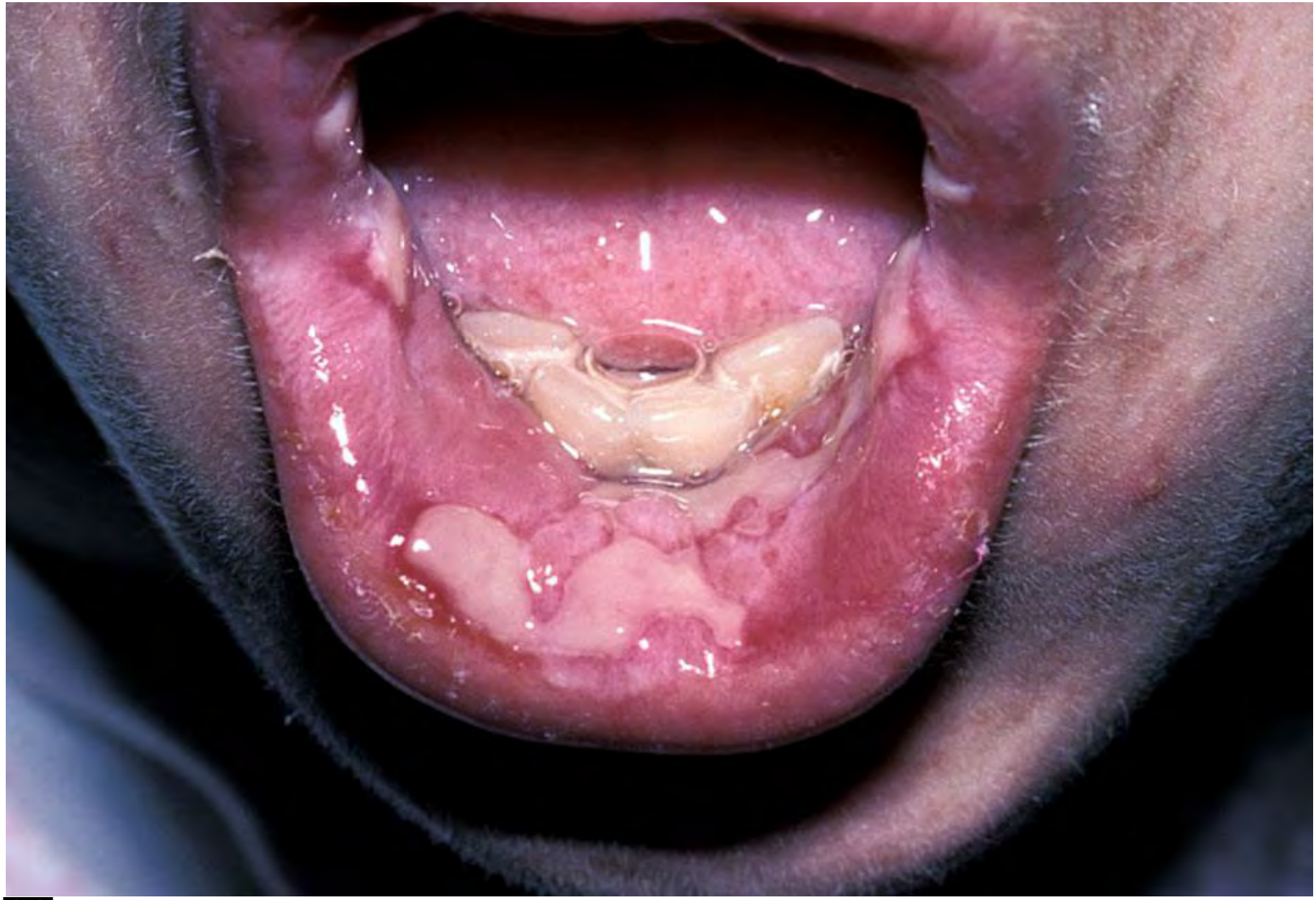
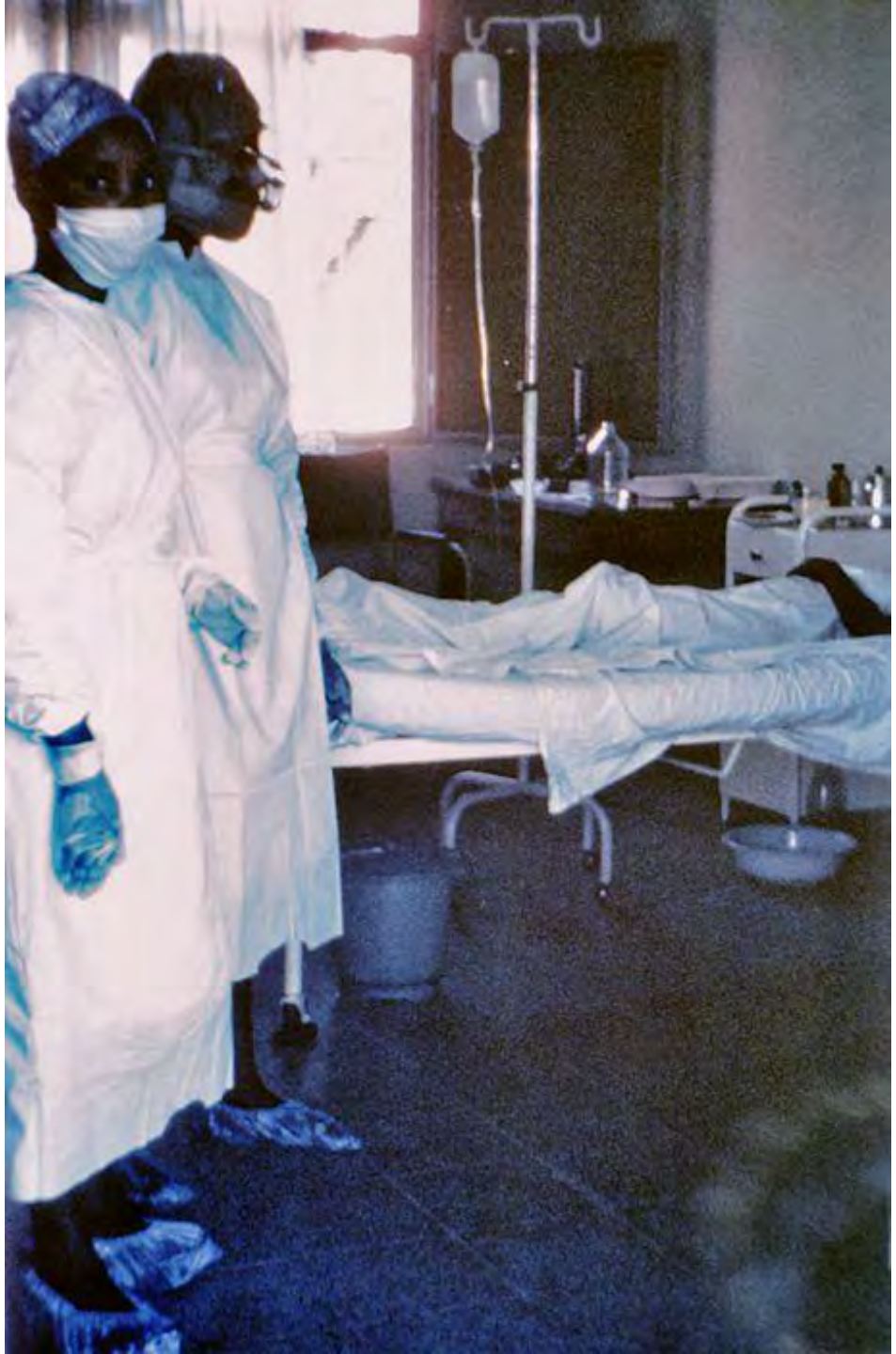
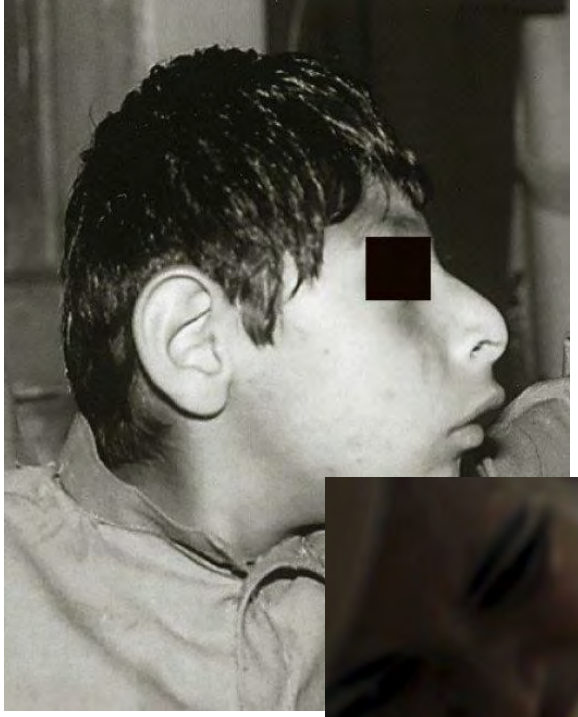
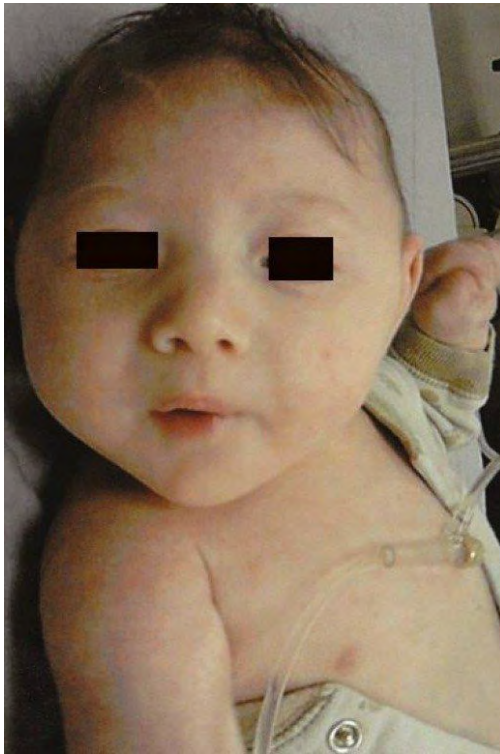
Acute and persistent virus life strategies

- No persistence in individual host
- Often disease associated
- High mutation rates (RNA viruses)
- Virus replicates in more than one species
- Little coevolution with host
- Horizontal transmission
- Highly dependent on host population structure
- Seldom evolves to persistence

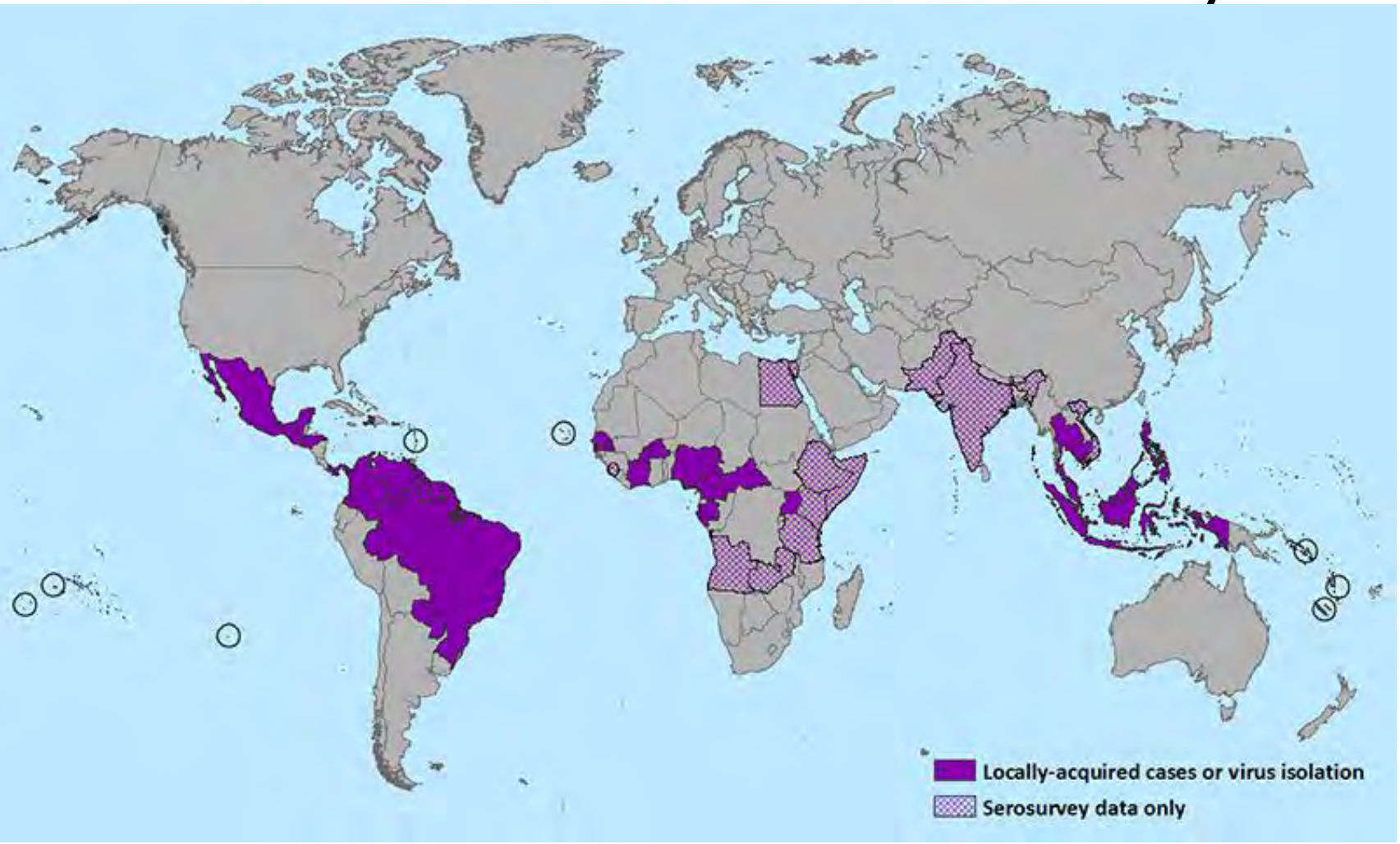








ZIKA VIRUS - Globally



47 countries in Americas involved

Sexual transmission in 5 countries

[CDC 2016]

Initial evidence of a link

- Rio de Janeiro
- 88 women enrolled with rash, usual symptoms prior 5d
  - ➢September 2015 to February 2016
  - ➢5 – 38 weeks gestation
  - ➢28% low grade fever
- 72 women (82%) tested positive in blood, urine or both
- Fetal ultrasound in 42 ZIKV +ve women (58%) and all 16 ZIKV-negative women
  - ➢Abnormalities in 12 / 42 (29%) and 0/16 negative

[Brasil 2016]

Table 2. Ultrasonographic Features of Fetuses and Findings at Birth.*

Fetus No.	Week of Gestation at Infection	Week of Gestation at Ultrasound Examination	Abnormal Findings on Doppler Ultrasonography	Findings at Birth
19	8	35	Microcephaly, cerebral calcifications, abnormal middle cerebral artery, intrauterine growth restriction	Microcephaly, cerebral calcifications on CT, global cerebral atrophy, macular lesions
40	8	20	Choroid plexus cyst, cerebellar atrophy (transverse diameter <5th percentile)	Still in utero
24	12	29	Microcephaly, cerebral calcification, Blake's cyst, agenesis vermis, club foot, intrauterine growth restriction	Still in utero
41	12	24	Mega cisterna magna (>95th percentile)	Still in utero
39	21	30	Cerebellar and cerebral right periventricular calcifications	Still in utero
17	22	26	Middle cerebral artery flow <5th percentile	Still in utero
12	22	27	Microcephaly, placental insufficiency as assessed by Doppler study, oligohydramnios, intrauterine growth restriction	Small for gestational age, head circumference proportional to body size, macular lesions
10	25	30	Normal first ultrasonogram, fetal death detected at 36 weeks on repeat ultrasonogram	Stillbirth
36	26	35	Microcephaly, abnormal umbilical artery flow (>95th percentile on the pulsatile index), intrauterine growth restriction	Small for gestational age, head circumference proportional to body size
38	27	35	Cerebral calcifications, ventriculomegaly, brachycephaly	Still in utero
2	30	34	None	Normal at birth
3	31	33	None	Normal at birth
53	32	38	Fetal death	Stillbirth
23	35	40	Anhydramnios, intrauterine growth restriction	Normal growth measure, poor sucking reflex, EEG abnormalities

* EEG denotes electroencephalogram, and CT computed tomography.

[Brasil 2016]

French Polynesian outbreak 2013

- 11.5% of population healthcare, 2.8% blood donors
- 2.3 / 1000 people had neurological complications
- 1.3 / 1000 (42 cases) GBS
  - 88% viral syndrome prior 23 d
  - 1 lab-confirmed during infection, several IgG positive
- Review post-Brazilian epidemic
  - 17 cases of malformations
  - None reported clinical symptoms
  - Serological review in 4 women showed IgG for Flavivirus
  - Possible asymptomatic ZIKV

[Paixao 2016]

Pregnancy recommendations

- No evidence that pregnant women are more susceptible or have a more serious disease (apart from possibly microcephaly in fetus/newborn)
- Consider postponing travel to infected areas
  - US recommends don't get pregnant for 6 months
  - El Salvador for 2 years
  - **Australian recommendations:**
- Pre-Travel advice Because Zika virus infection in a pregnant woman may cause severe birth defects, deferral of travel to High Risk countries for these women is recommended. If the woman does decide to travel, discussion with a doctor about preventing Zika virus transmission from mosquitoes and sexual partners is advised.
- For Moderate risk countries, a pregnant woman should consider deferring travel, based on her individual risk assessment. Recommendations for pregnant women planning to travel to Low risk countries should be based on an individual risk assessment.
- Women planning a pregnancy should either defer travel as described above, or defer pregnancy.

Compatible illness whilst pregnant

- Recommendations for testing from DoH based on time since contracting the disease
- Within 2 weeks of travel to an area then test ZIKV
  - Consider CHIKV, DENV
  - Presence in semen up to 24 wks
- Amniocentesis (0.1% fetal loss if < 24 wga)
  - RT-PCR not validated, ?sensitivity / specificity
- Evaluate fetus and infants of women infected for possible congenital infection
  - Serial US every 3 – 4 weeks to monitor growth if lab evidence in serum or amniotic fluid
- Report cases to local health authorities

Men and pregnancy

- Men who reside in or have travelled to an area of active disease who have a pregnant partner should abstain or use condoms for the duration of the pregnancy
- Men and non-pregnant partners who have travelled or resided in an affected area could abstain or use condoms
  - ➢ It is acknowledged that a majority of people will have an asymptomatic disease

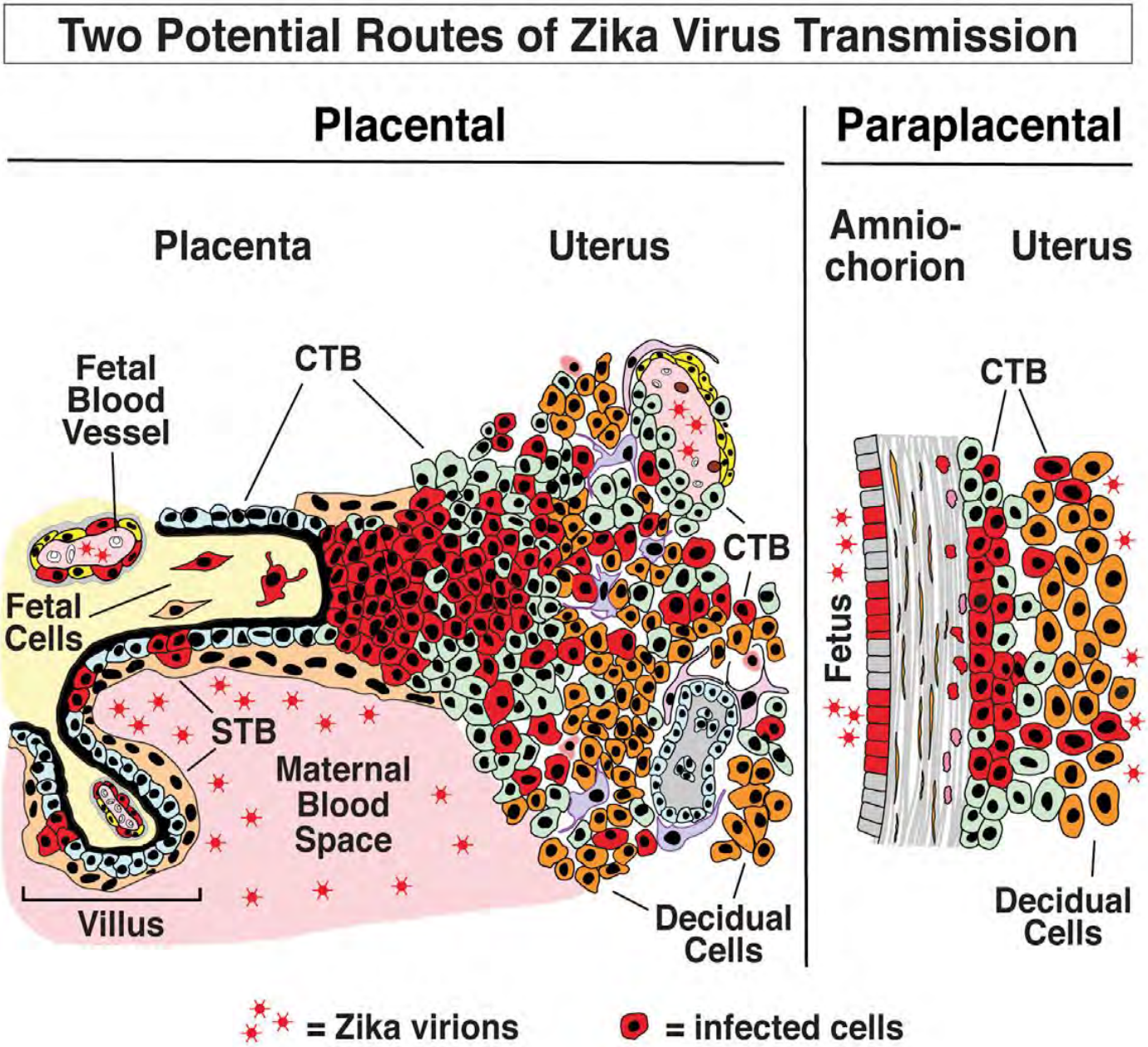
http://www.cdc.gov/mmwr/volumes/65/wr/mm6503e1.htm

ZIKV in the Placenta

- 88 pregnant women with rash <38wga (Brasil 2016)
  - 82% ZIKV infn
  - 12/42 (27%) abnormal
  - No ZIKV infn, 0/16 abnormal
- Microcephaly, 24/32 mothers ZIKV infn vs 39/61 controls (Araujo 2016)

- ZIKV infn of placenta
  - T2 infects villi cytotrophoblasts, endothelial, fibroblasts
  - Amniochorionic membranes amnion epithelial cells, trophoblast progrenitors
  - Receptors and cell entry cofactors (Y kinases) likely impt Axl, Tyro3, Mertk

[Tabata 2016]


Two Potential Routes of Zika Virus Transmission
Placental
Paraplacental
Placenta
Uterus
Amnio-
chorion
Uterus
Fetal
Blood
Vessel
CTB
CTB
CTB
Fetal
Cells
STB
Maternal
Blood
Space
Villus
Decidual
Cells
Fetus
Decidual
Cells
= Zika virions
= infected cells

[Tabata 2016]

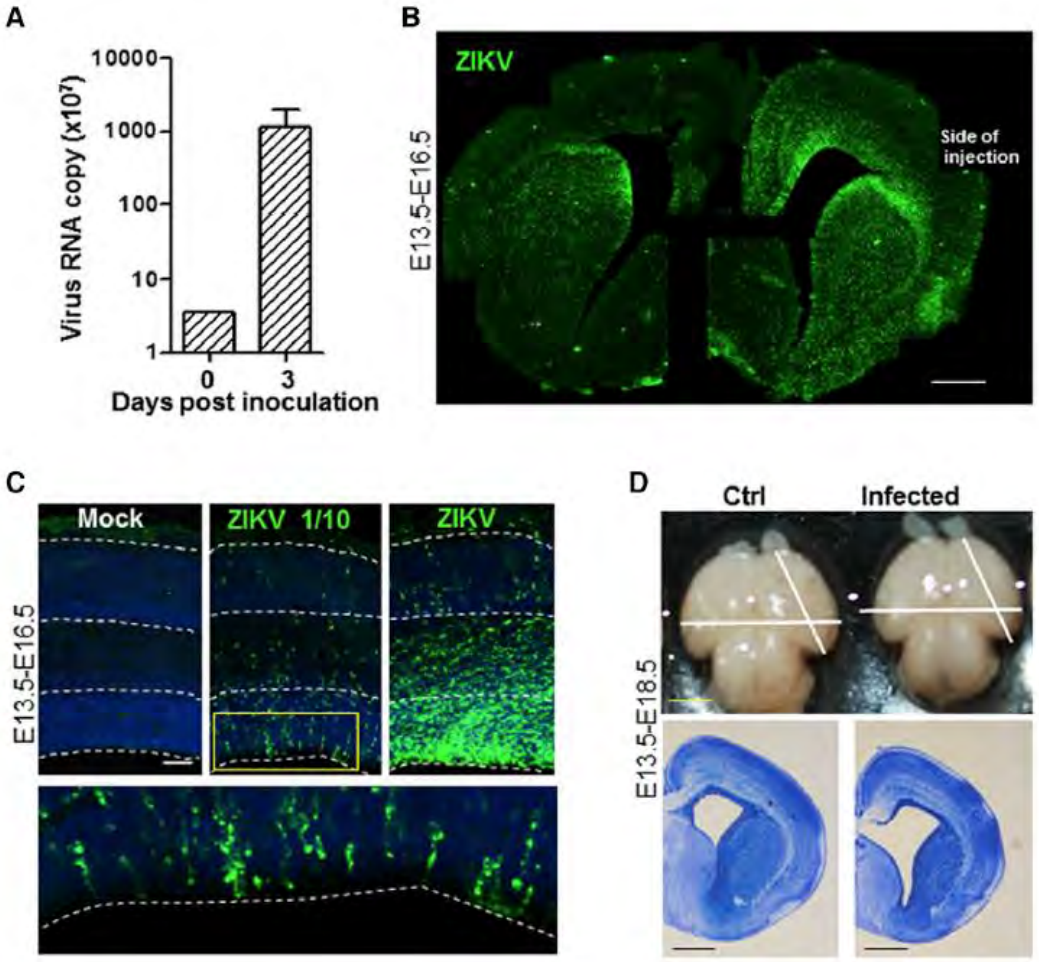


Figure 1. ZIKV Replicates Efficiently in Embryonic Mouse Brain and Causes Microcephaly

(A) Viral RNA copies were determined by real-time RT-PCR of whole brains. Data are means ± SEM from two independent experiments.

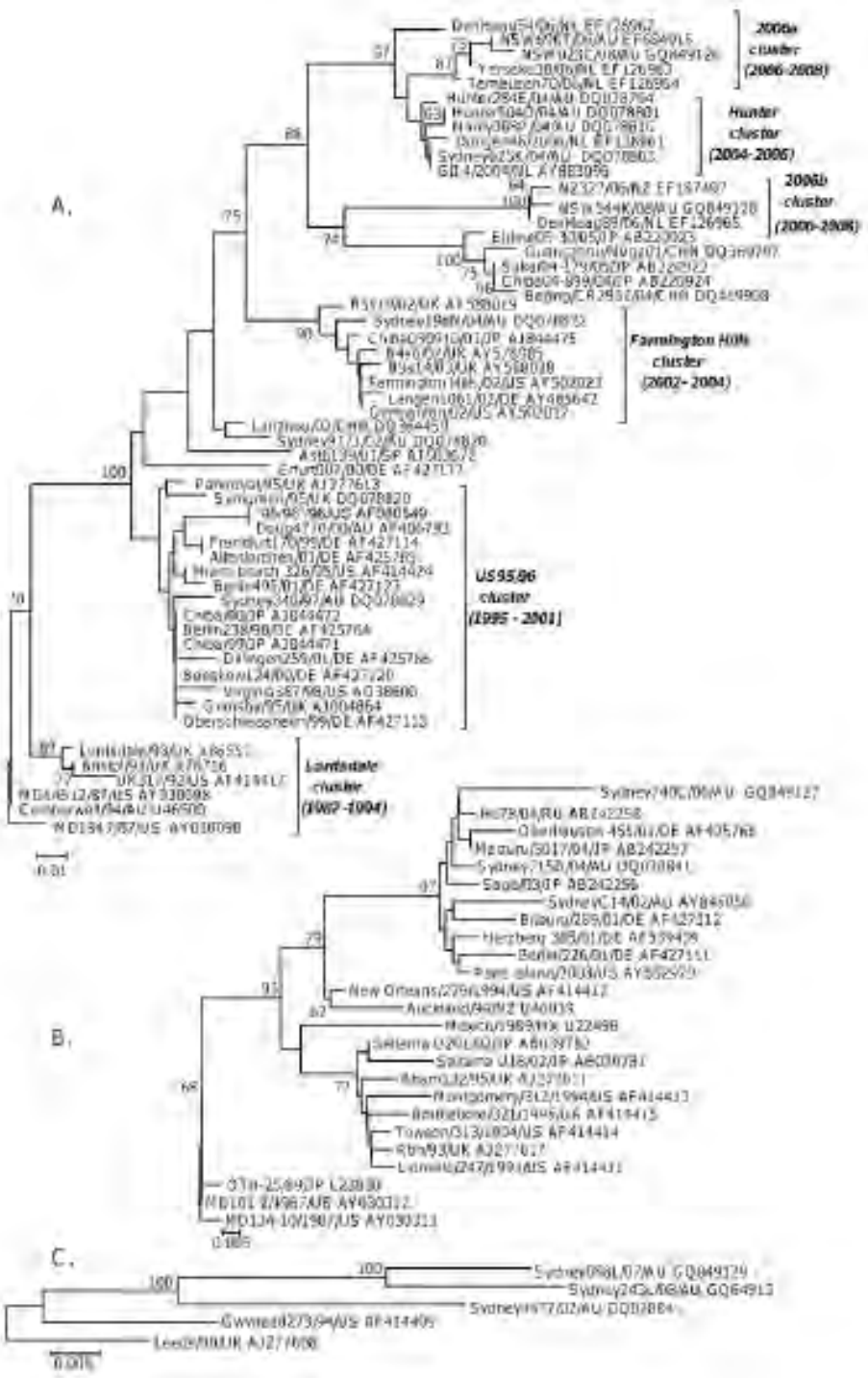
(B–D) Embryonic brains were injected with ZIKV in the lateral ventricle at E13.5 and inspected at E16.5 (B and C) or E18.5 (D). (B) Coronal section of a whole brain slice stained with ZIKV serum. Scale bar, 400 μm. (C) Images of coronal sections stained with DAPI to label nuclei (blue) and ZIKV antiserum (green). Left: mock-infected (control), middle: 1/10 diluted, right: undiluted virus. Lower panel: High-magnification of the area outlined by the yellow box. Scale bar, 40 μm. (D) Images of E18.5 infected or mock-infected littermate brains. Lower panel: similar position of coronal sections of cortices with Nissl staining. Scale bar, 5 mm (upper panel), 1 mm (lower panel).

See also Figure S1.

ZIKV Infects NPCs and Causes Cell Death

Increased cell death has been shown as one of the causes of microcephaly (Xu et al., 2014). In order to determine whether cell death contributes to the smaller size of infected brains, we infected or mock-in-

[Li 2016]



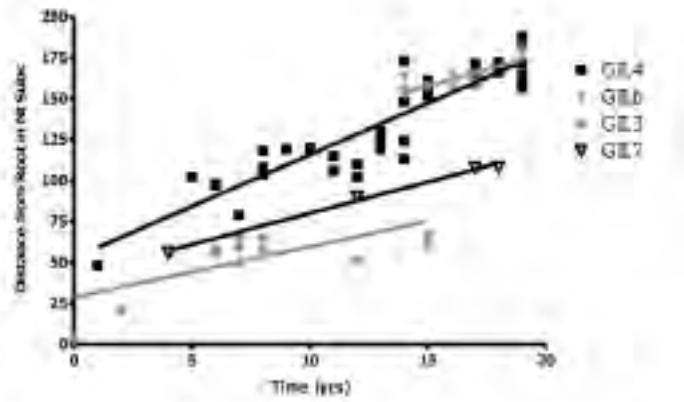


Figure 4. Rate of evolution for the GII.4, GII.7, GII.b/GII.3 and GII.3 strains. The rate of evolution for each genotype was determined

OPEN ACCESS Freely available online

PLoS PATHOGENS

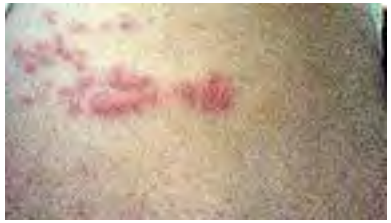
Rapid Evolution of Pandemic Noroviruses of the GII.4 Lineage

Rowena A. Bull[1], John-Sebastian Eden[1], William D. Rawlinson[1,2], Peter A. White[1]*

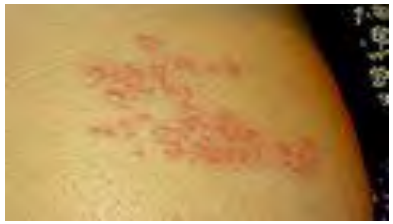
1 School of Biotechnology and Biomolecular Sciences, Faculty of Science, University of New South Wales, Sydney, New South Wales, Australia, **2** Virology Division, SEALS, Department of Microbiology, Prince of Wales Hospital, Randwick, Sydney, New South Wales, Australia

Acute and persistent virus life strategies

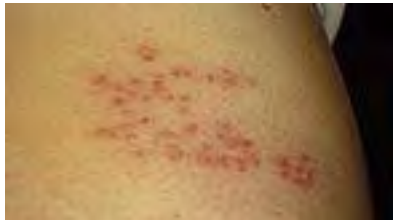
- Persistent in individual host
- Acute disease often inapparent
- Genetically stable
- Highly species specific
- Coevolution with host
- Transmission is often from parent to offspring (vertical) or through sexual contact
- Less dependent on host population structure
- Often the source of emerging acute disease in new host species



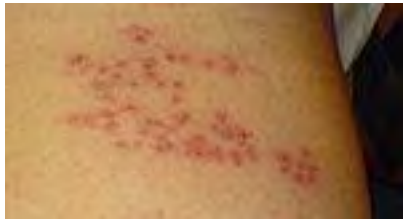
Day 1



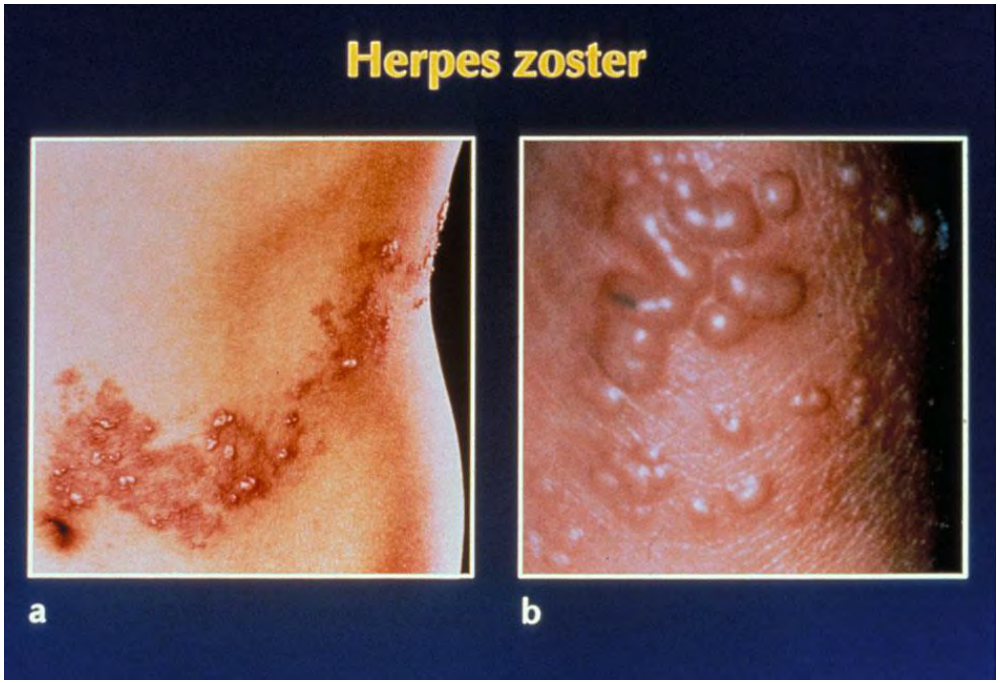
Day 2



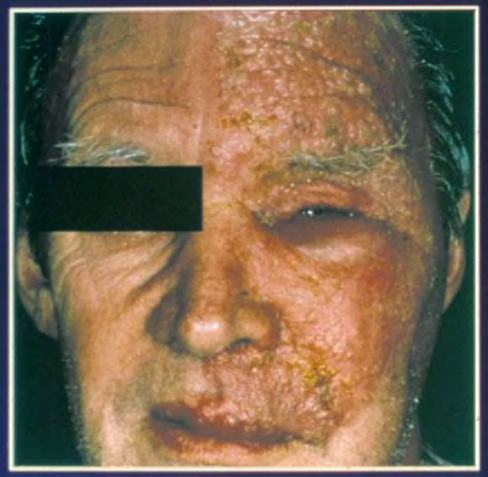
Day 5



Day 6



Herpes zoster



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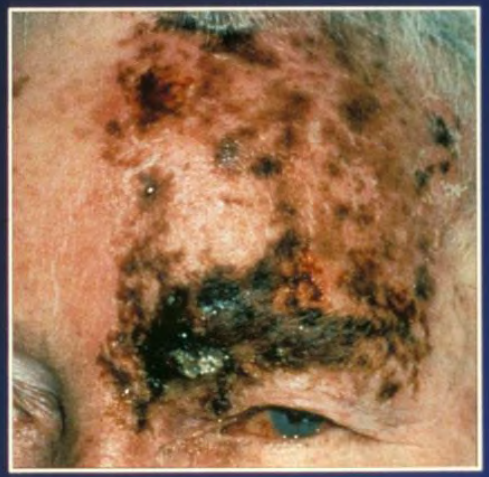

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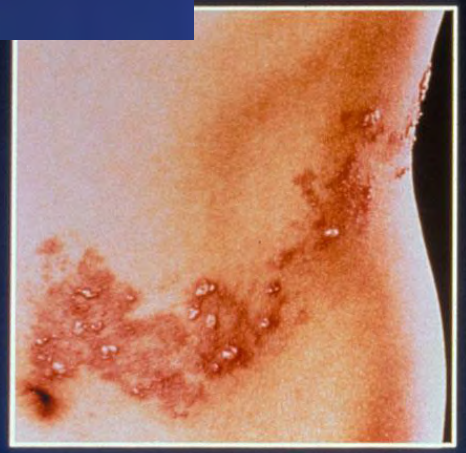
Herpes zoster



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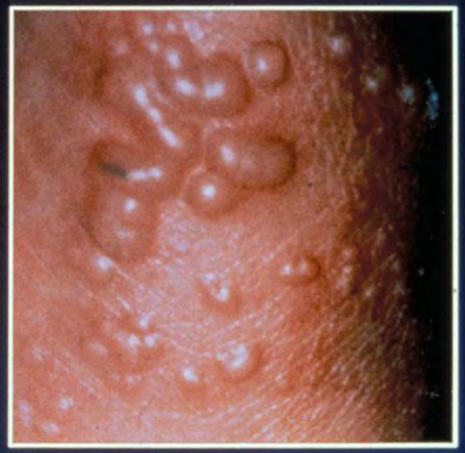

b

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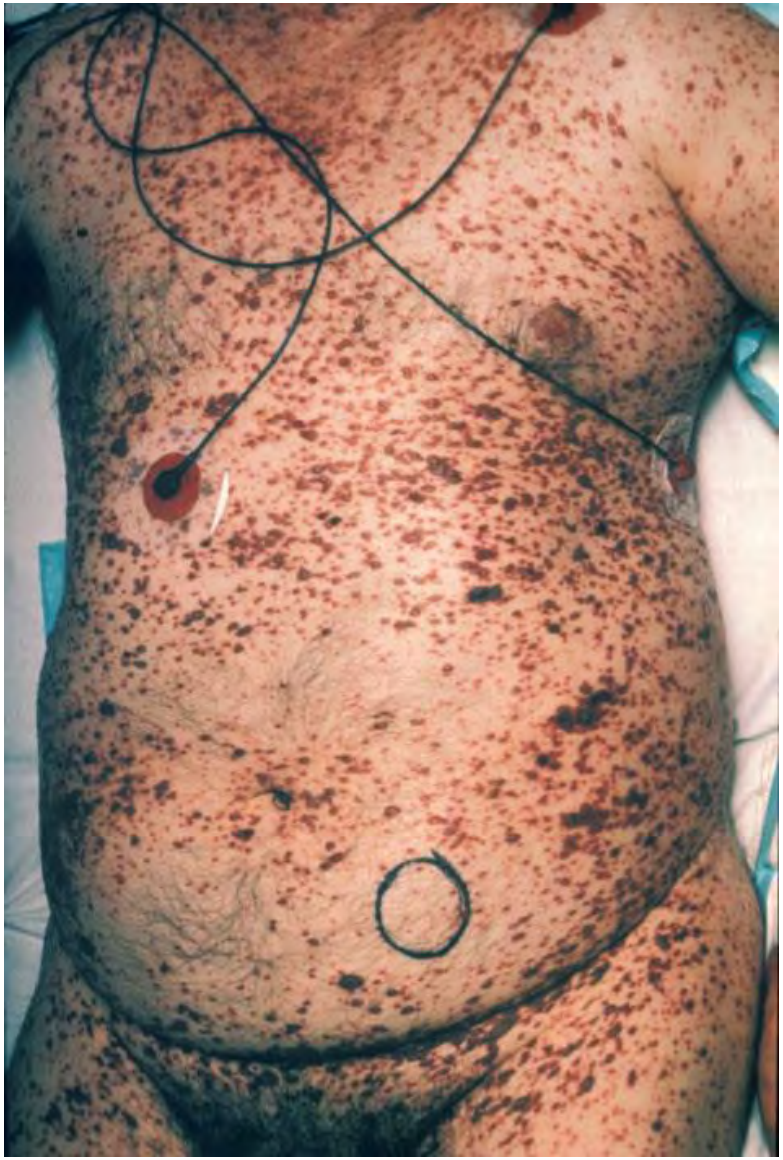

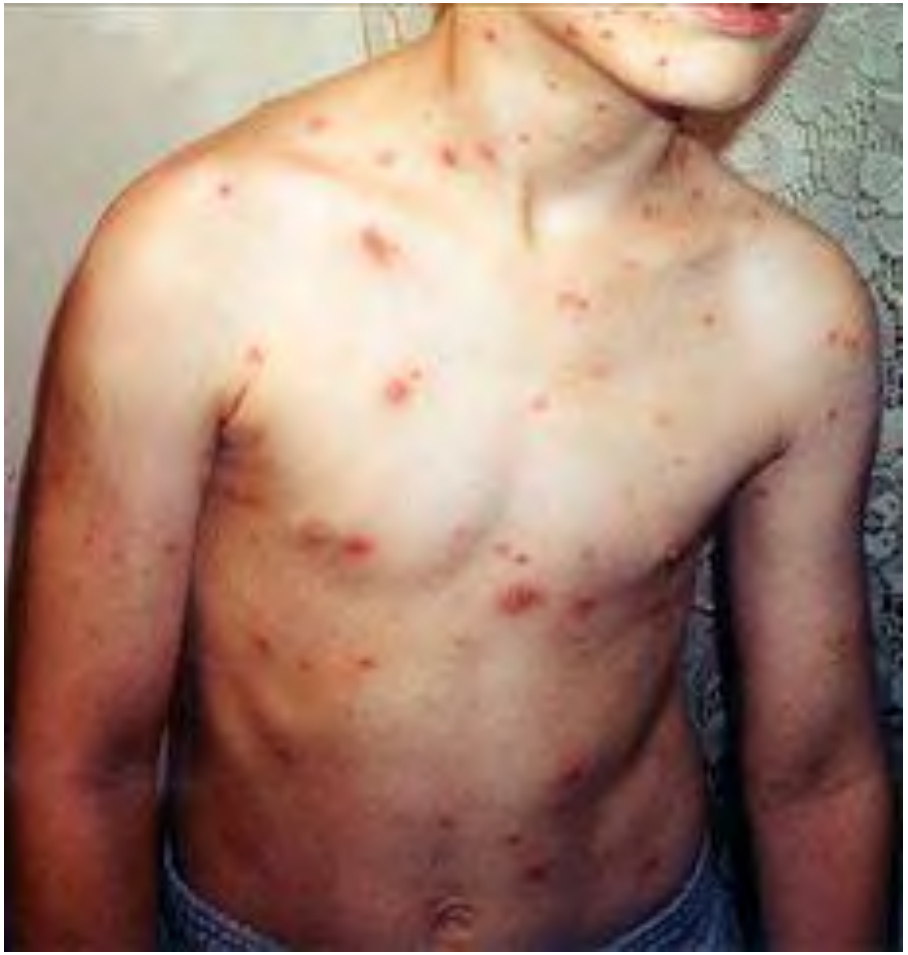

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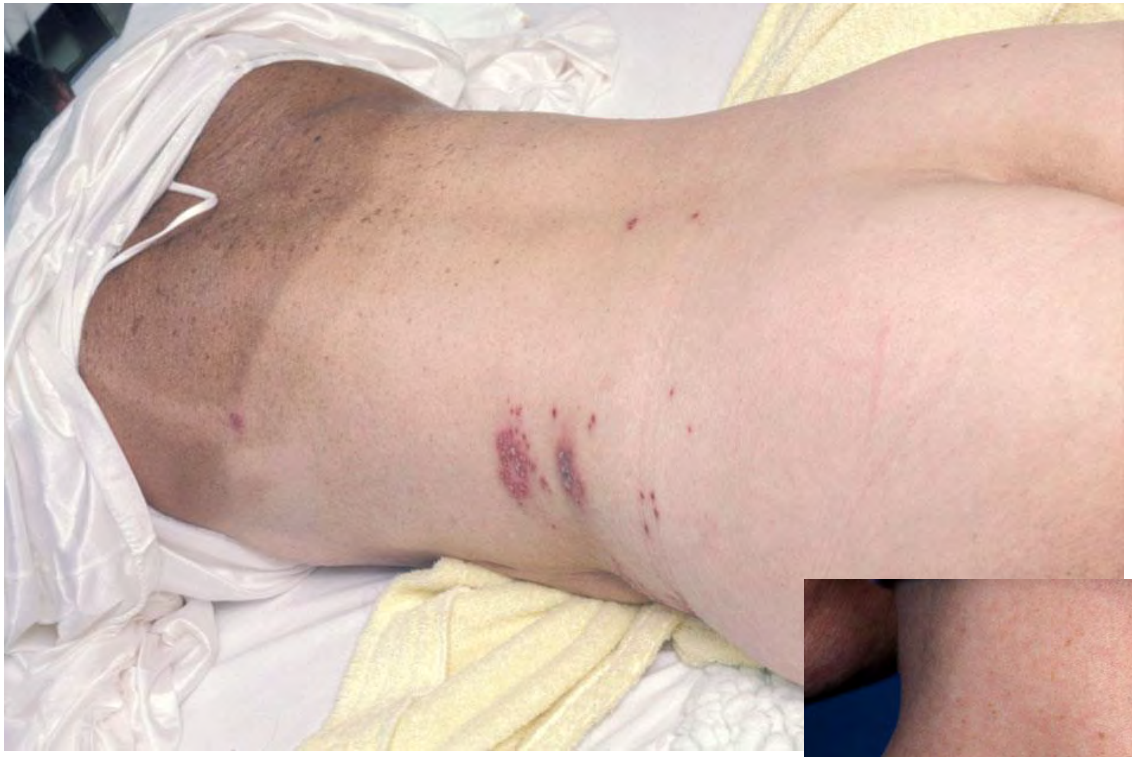

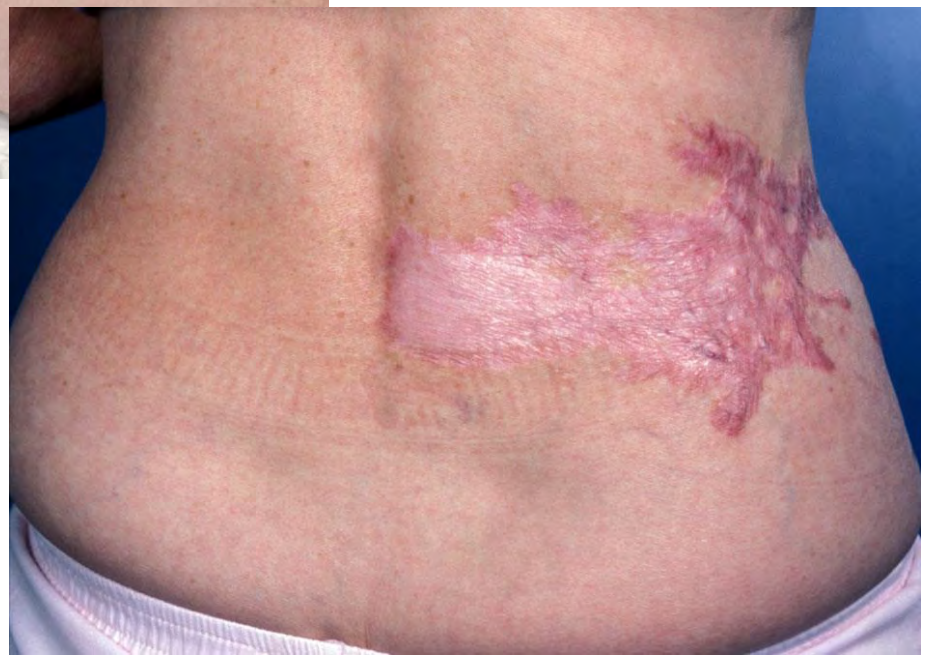

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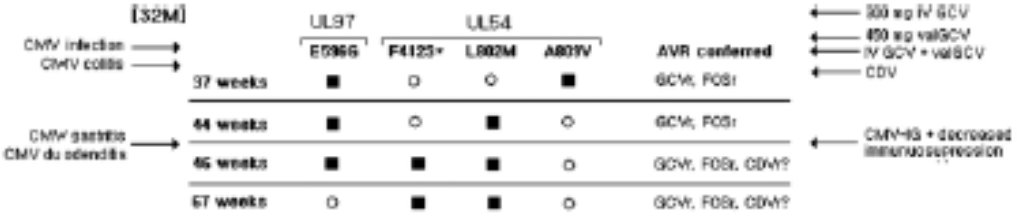

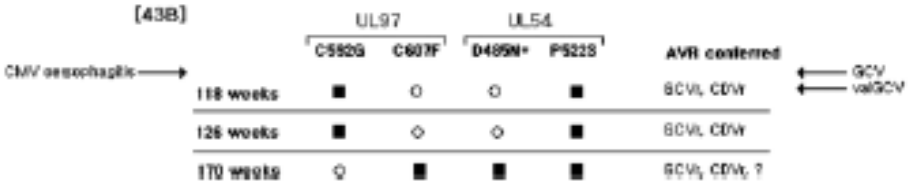

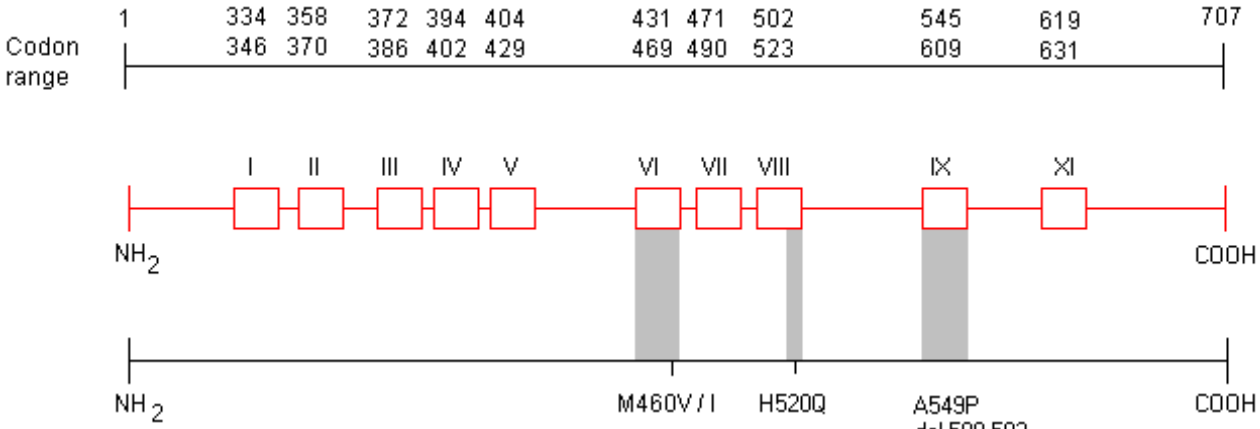

Diversity of antiviral-resistant human cytomegalovirus in heart and lung transplant recipients

J.M. Iwasenko, G.M. Scott, Z. Naing, A.R. Glanville, W.D. Rawlinson. Diversity of antiviral-resistant human cytomegalovirus in heart and lung transplant recipients.
Transpl Infect Dis 2010.

Abstract: Immunocompromised transplant recipients are at high risk for human cytomegalovirus (CMV)-related infection and disease. Antiviral prophylaxis and treatment have reduced CMV morbidity and mortality, but at times promote development of antiviral-resistant CMV strains that can significantly contribute to adverse clinical outcomes in transplant recipients. We have investigated CMV genotypes in transplant recipients (bone marrow, stem cell, kidney, heart, lung, and

J.M. Iwasenko[1,2,4], G.M. Scott[1,2], Z. Naing[1], A.R. Glanville[3,4], W.D. Rawlinson[1,2,4]
[1]Virology, Department of Microbiology, South Eastern Area Laboratory Services (SEALS), Prince of Wales Hospital, Randwick, New South Wales, Australia, [2]School of Biotechnology and Biomolecular Sciences, Faculty of Science, University of New South Wales, Kensington, New South Wales, Australia, [3]Lung Transplant Unit, St Vincent's Hospital, Darlinghurst, New South Wales, Australia, [4]School of Medical Sciences, Faculty of Medicine, University of New South Wales, Kensington, New South Wales, Australia

OUTLINE

1. What is a virus
   - Characteristics
   - Structure
   - Replication
   - Definitions
2. How viruses cause disease
   - Molecular principles
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Diagnostic Methods

- Serology - retrospective
- Ag
  - Protein based
  - IFA (Respiratory)
  - WB (HIV)
  - Protein function (HIV-RT)
- Culture – some viruses non-cultivable
- Molecular
  - Virion nucleic acid
  - HIV RNA, HCV RNA
  - CMV DNA
- Detection of viruses in different/new situations
- Emerging Microarray different formats, HPLC, Protein amplification, MALDI-TOF
- Deep sequencing and multiple sequence determinations

ZIKV

- Arbovirus with sexual spread
- Usually minor illness, asymptomatic
  - Rarely GBS
  - Pregnant women no increased risk
  - Fetal infection microcephaly
  - Transplacental spread
- Australian transmission not recorded but *"There is a risk of transmission of Zika virus from infected returning travellers in areas of North Queensland where a suitable vector (Aedes aegypti) exists and is currently considered dengue receptive."* (DOH URL)

LOU COLLECTION/ALAMY

This 22-year-old is severely disabled by fetal cytomegalovirus infection and cannot communicate verbally.

PUBLIC HEALTH

Zika raises wider birth-defect issue

Cytomegalovirus is a greater global problem than Zika.

BY DECLAN BUTLER

many more are born with serious defects. The causes are many — some known, some not. A lung or spleen damage, or neurological problems including developmental disability or loss of hearing or sight.

CMV's link to birth defects has been known since the 1950s — yet a 2012 survey found that only 13% of US women and 7% of men had heard of congenital CMV (M. J. Cannon *et al. Prev. Med.* **54**, 351–357; 2012). Low awareness is deadly, says Gail Harrison, an infectious-disease researcher in CMV at the Baylor College of Medicine and the Texas Children's Hospital, both in Houston.

There is no vaccine, so precautions — handwashing and avoiding contact with children's saliva and urine — are the only defence. Harrison works closely with patient groups to promote awareness, but says that she struggles with the inertia of state and federal agencies in helping to get these messages across.

The administration of US President Barack Obama has requested more than US$1 billion for research and control measures for Zika, and the website of the US Centers for Disease Control and Prevention (CDC) is awash with information and advice on that virus, she notes. But the more modest amount of information on CMV has to be actively searched for.

Leading health experts and the CDC expect that Zika in the United States will be limited to small, localized outbreaks in southern states where *Aedes aegypti*, the mosquito that transmits the virus, is present during warm parts of the year. That prediction is based on the pattern of past US outbreaks of dengue and chikungunya, two other diseases carried by the same mosquito. For the United States, says

[Nature July 2016]

VECTOR-BORNE DISEASES

OPINION

Zika virus — reigniting the TORCH

Carolyn B. Coyne and Helen M. Lazear

Abstract | The recent association between Zika virus (ZIKV) infection during pregnancy and fetal microcephaly has led to a renewed interest in the mechanisms by which vertically transmitted microorganisms reach the fetus and cause congenital disease. In this Opinion article, we provide an overview of the structure and cellular composition of the human placenta and of the mechanisms by which traditional 'TORCH' pathogens (*Toxoplasma gondii*, other, rubella virus, cytomegalovirus and herpes simplex virus) access the fetal compartment. Based on our current understanding of ZIKV pathogenesis and the developmental defects that are caused by fetal ZIKV infection, ZIKV should be considered a TORCH pathogen and future research and public health measures should be planned and implemented accordingly.

placenta, the mechanisms by which other microorganisms that are associated with congenital disease breach the placental barrier and the current models to study ZIKV pathogenesis both *in vitro* and *in vivo*. What we know about ZIKV suggests that it should be classified as a TORCH pathogen, so that the experience with traditional TORCH pathogens can inform future research and public health priorities regarding ZIKV.

The placental barrier

To reach the human fetus, ZIKV must overcome the barrier presented by the placenta, which develops within days of conception and is indispensable for pregnancy.

In the early stages of pregnancy, the human placenta is responsible for anchoring the blastocyst to the maternal endometrium

[Coyne Nov 2016 Nat Rev Micro]

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Viral Syndromes

- Adenopathy and glandular fever
- Arthritis
- Carditis
- Chronic Fatigue Syndrome
- Congenital and perinatal disease
- Exanthemata and skin disease
- Eye disease
- Gastroenteritis

Viral Syndromes

- Haemorrhagic fevers
- Hepatitis
- Immunocompromised infections
- Neurological disease
  - encephalitis and meningitis
- Pancreatitis and diabetes
- Respiratory disease
- Sexually Transmitted Infections (STD, STI)

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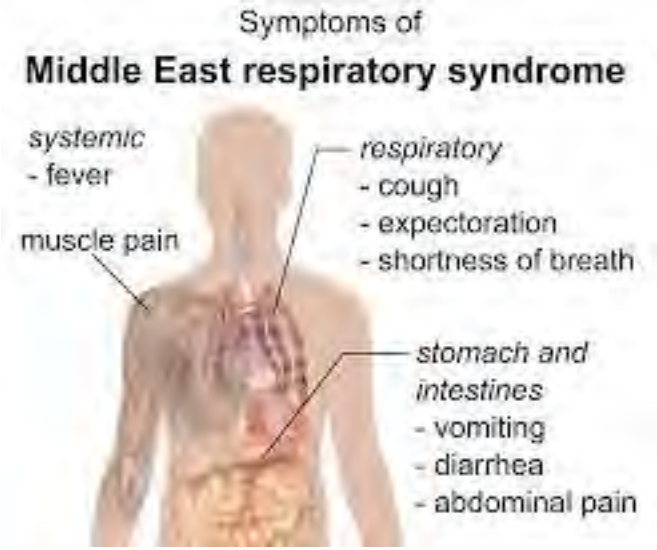
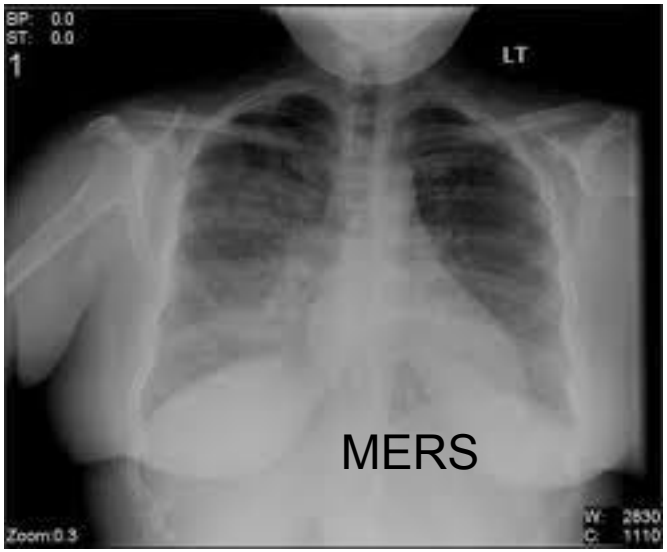
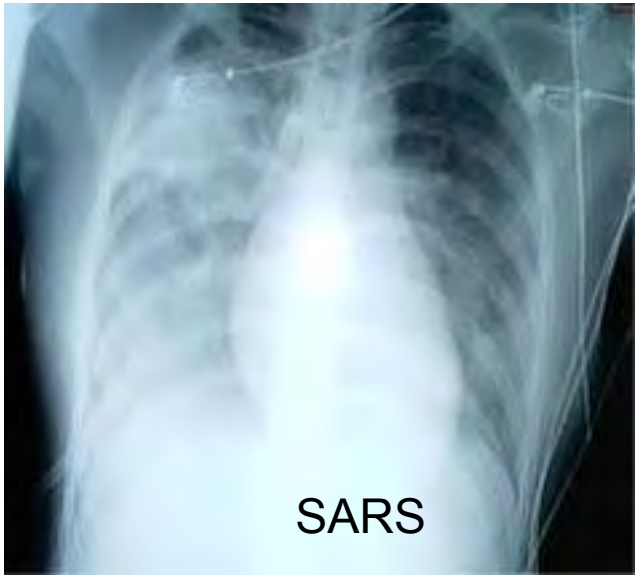
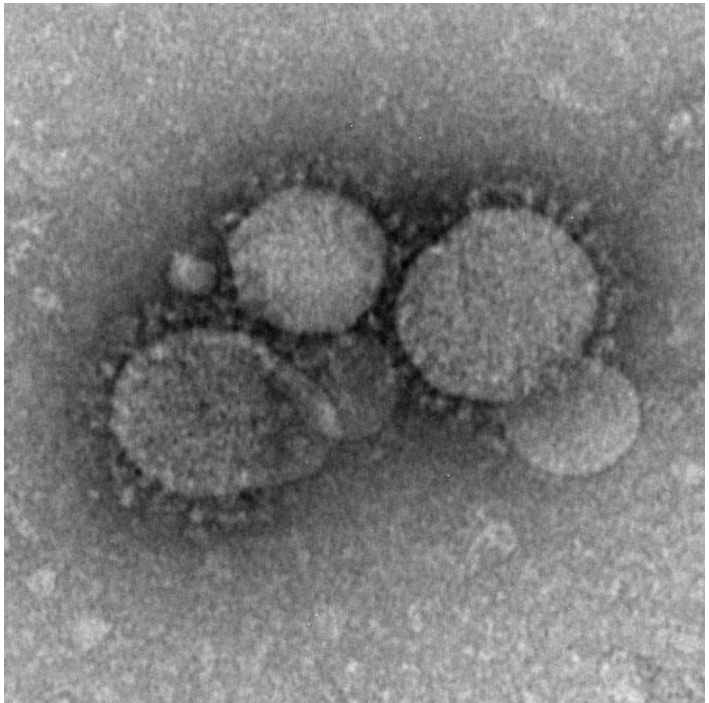
ACUTE PNEUMONITIS









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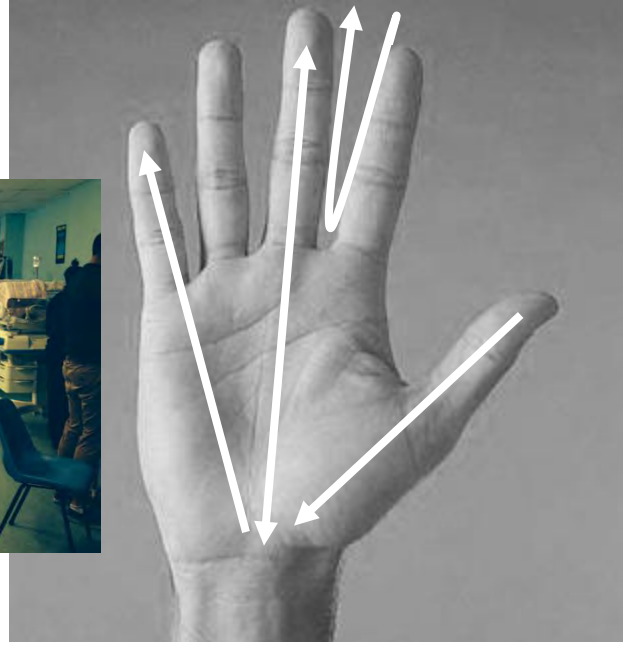
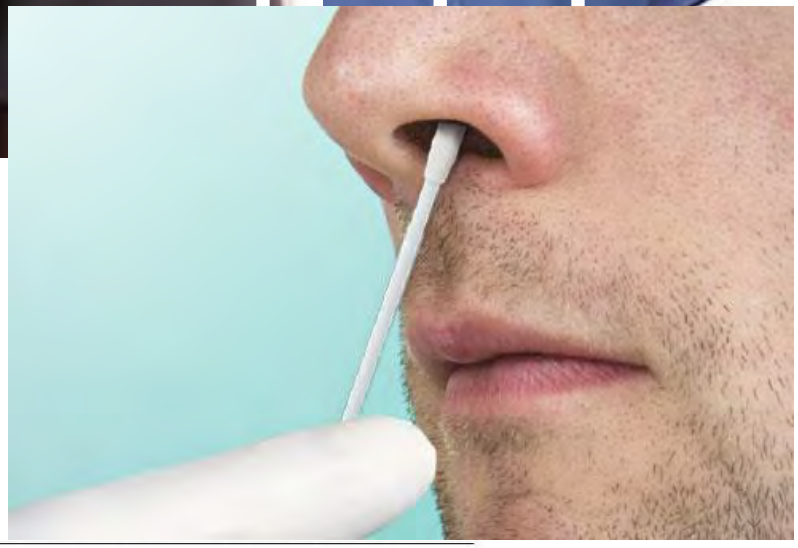




- Sampling days: once every week for 8 weeks
- Sampling procedure: nasal, hand and personal clothing specimens
- Environmental samples :"point prevalence"

Respiratory Syncytial Virus is Present in the Neonatal Intensive Care Unit

Nusrat Homaira,[1] Joanne Sheils,[2] Sacha Stelzer-Braid,[3,4] Kei Lui,[1,2] Ju-Lee Oie,[2] Tom Snelling,[5] Adam Jaffe,[1] and William Rawlinson[3,4,6]

[1]*School of Women's and Children's Health, UNSW, Randwick, Australia*
[2]*Department of Newborn Care, Royal Hospital for Women, Randwick, Australia*
[3]*Serology and Virology Division (SAViD), SEALS Microbiology, Prince of Wales Hospital, Randwick, Australia*
[4]*School of Medical Sciences, UNSW, Australia*
[5]*Telethon Institute for Child Health Research, Institute for Child Health Research, University of Western, Australia*
[6]*School of Biotechnology and Biomolecular Sciences, UNSW, Australia*

Longitudinal Prospective Study

- **Identify potential sources of RSV transmission within neonatal intensive care unit (NICU)**
- Study site: NICU of Royal Hospital for Women, Randwick

- Study duration: May-June 2014
- Study participants:
  - All health personnel
  - Every third neonate and their visitors
  - Any child clinically suspected with a RSV infection

Characteristics	Visitors N=80 n(%)	HCWs N=84 n(%)
Sex (female)	54 (69%)	78 (93%)
Number of swabs collected	160 of each	170 of each
Swabs positive		
Nose	**1 (1%)**	**1 (1%)**
Hand	**0 (0%)**	**0 (0%)**
Dress	**4 (4%)**	**0 (0%)**

Dress and nasal swab specimens positive from one of the visitors

15 month old sibling

None of the RSV positive participants had preceding respiratory symptoms

None of the positive participants were related

None of the hand specimens were positive

Overall high CT value

Environmental Samples

- 34 specimens
  - Bed rails, chairs, bed surfaces, counter tops of the sampled children
  - Nurse's station: table, computer and chair
  - Doctors Station: table, computer and chair
  - One bed surface and one chair adjacent to a patient positive
  - Computer on nurses' desk positive

Viruses in childcare settings

TABLE II. Predictors of Symptomatic Illness and hRV Detection

Outcome construct	Overall corrected % predicted	Nagelkerke R^2	Significant predictor constructs	Odds ratio (95% CIs)	*P*-value
Detection of hRV RNA on clothing (evening sample)	97.1	0.233	Detection of hRV RNA on clothing (morning sample)	39.7 (8.5–185.6)	<0.001
Detection of hRV RNA on clothing (morning or evening sample)	95.2	0.112	Self-recognition of symptomatic illness, 2 days previously	8.2 (1.3–53.1)	0.028
			Worked at center 2	0.07 (0.07–0.6)	0.018

Journal of Medical Virology 87:925–930 (2015)

Personal Clothing as a Potential Vector of Respiratory Virus Transmission in Childcare Settings

Jan Gralton,[1,2] Mary-Louise McLaws,[1] and William D. Rawlinson[2,3,4]*
[1]UNSW Medicine, UNSW Australia, Australia
[2]Virology Division, Prince of Wales Hospital, Australia
[3]School of Medical Sciences, UNSW Australia, Australia
[4]School of Biotechnology and Biomolecular Sciences, UNSW Australia, Australia

CMV in childcare settings

Risk

- Infection through high levels of CMV in saliva and urine in children
- Exposure of childcare staff through changing nappies, feeding, contaminated environmental surfaces
- higher rates of paediatric shedding in childcare (50% in childcare versus 20% in controls in Emergency for subjects 3 months to 6 years age) [Grosjean 2014]

CMV excretion among childcare staff from two childcare centres in Sydney

- 114 clothing samples + 125 nasal samples from 20 trained childcare staff, Sydney 5 weeks in 2011
- 6/125 nasal swabs +ve CMV DNA
- CMV excretion rate 30% (6/20) among childcare staff
- 0/114 clothing swabs +ve [van Zuylen 2017]

Things we knew for certain

- HCV causes chronic disease -> DAA
- Herpesviruses exist as single strains

->Quasispecies data

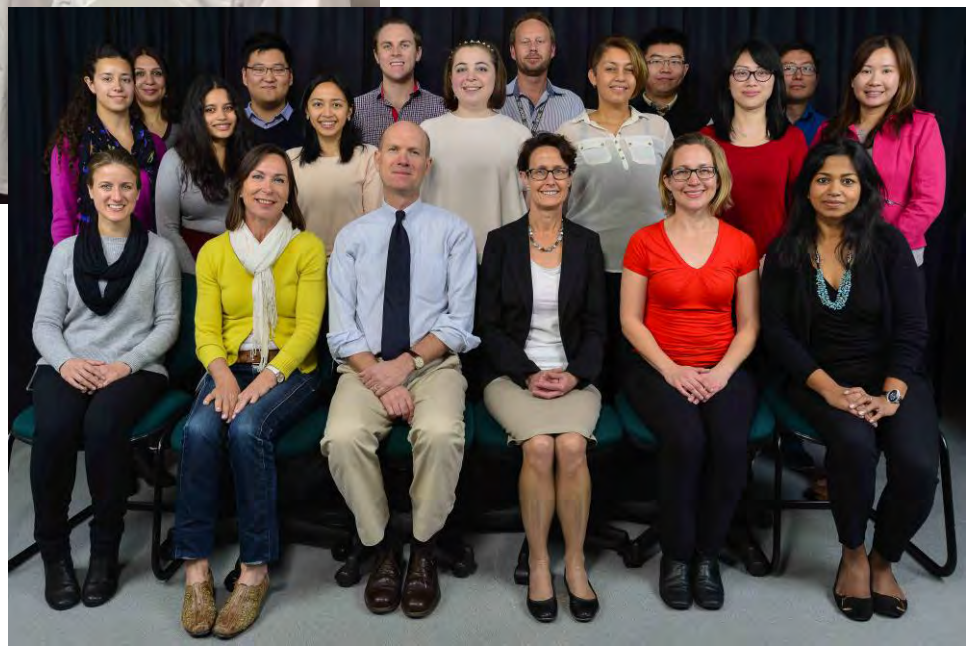
- Conservative presidential candidates could get no more bizarre ->




There is no better idea of rationality than that of a readiness to accept criticism; that is criticism which discusses the merits of competing theories from the point of view of the regulative idea of truth

Karl Popper 1992


know pathology
know healthcare



Viruses in May 2017

Thank you to our participants and our sponsors

Roche

DiaSorin The Diagnostic Specialist

RCPA The Royal College of Pathologists of Australasia

Abbott Molecular

RCPAQAP RCPA Quality Assurance Programs

HOLOGIC® The Science of Sure

SEALS South Eastern Area Laboratory Services

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Background

- "Colds" trigger asthma exacerbations (1927)
- Multiple "triggers" for asthma symptoms
  - allergens, smoke/pollution, Weather
  - Infection
- Other factors increase symptoms with colds
  - allergic, allergen exposure, ?low Vit D
- Childhood rhinovirus hRVC > A > B > others
- Back to school asthma - more & different viruses
- Infants, severe RSV+ hRV increases risk of asthma in childhood 5-10 X, typically allergic children

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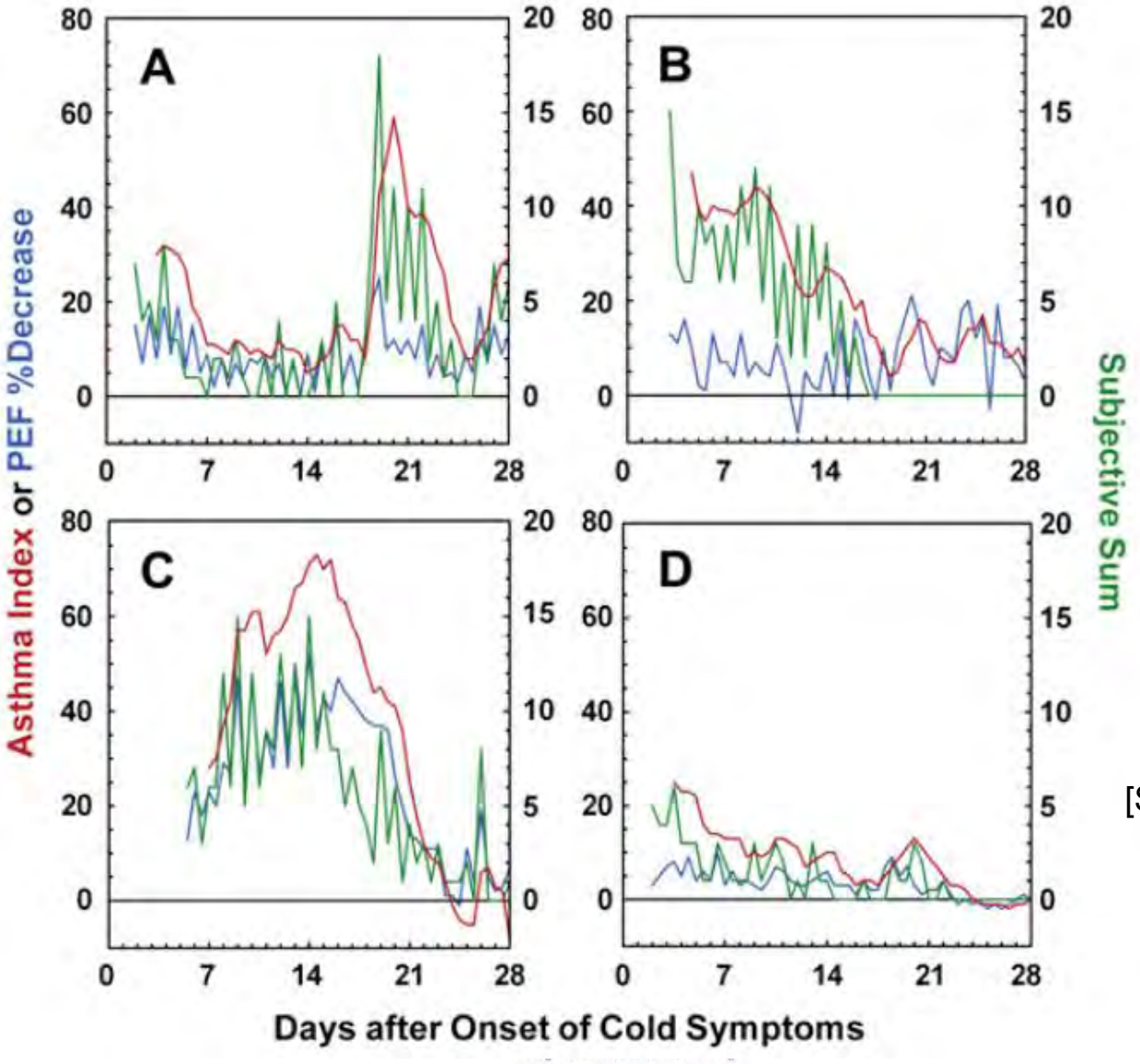
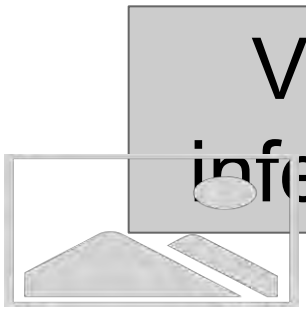
Asthma symptoms may not follow a predictable pattern after natural viral infections



PEFR fall – blue
Symptoms – green
Asthma index - red

[Sorkness, JACI 2008]

Project background



Virus infection

hma
erbation

WOOLCOCK INSTITUTE of MEDICAL RESEARCH

STUDY: Asthma Viruses Environment factors

Exploring the role of respiratory virus infections in childhood asthma exacerbations

NHMRC Project Grant 633238

CIs: A: Dr Euan Tovey, B: Prof Guy Marks, C: Dr Brian Oliver, D: Prof William Rawlinson
E: A/Pr Helen Reddel, F: Prof Wayne Smith

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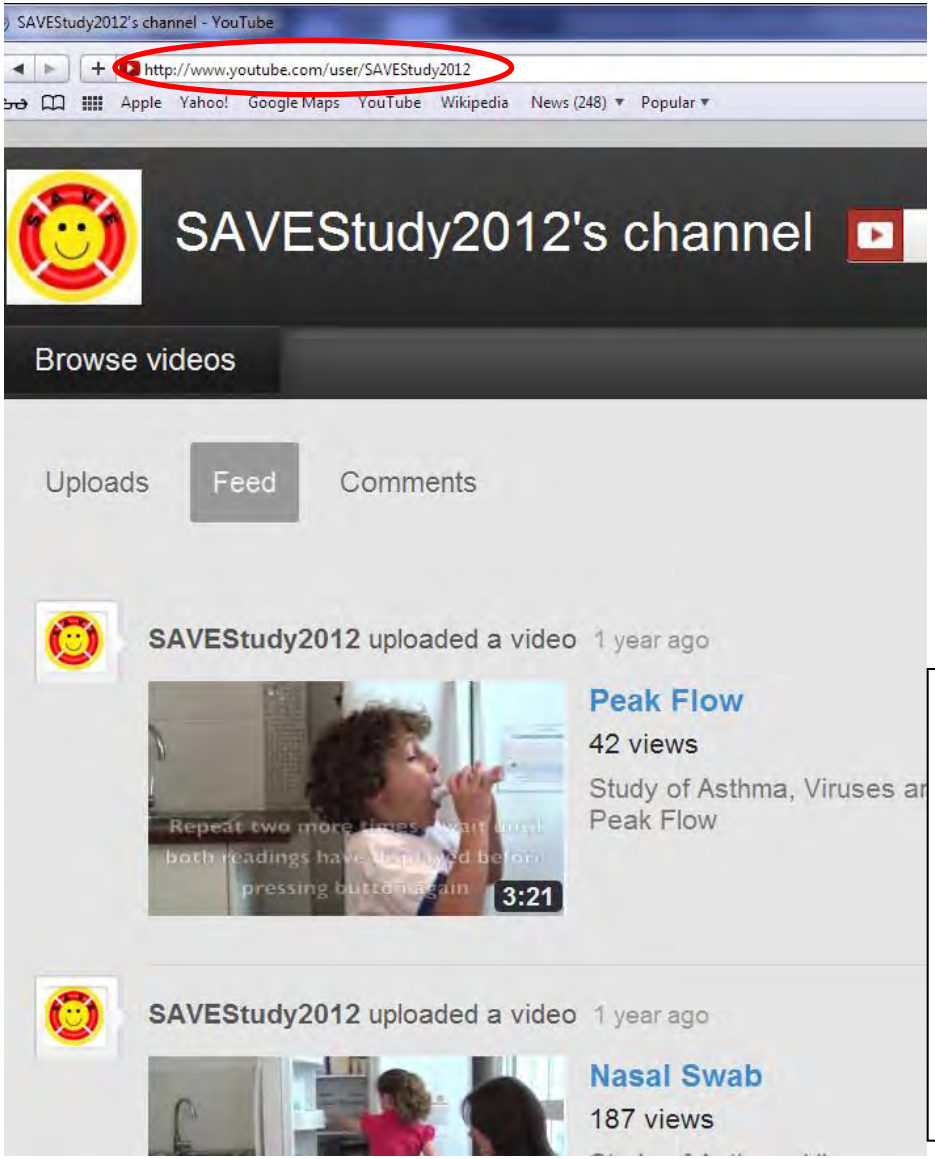

4 x 100 µl of FESS saline per nostril and then blow into bag

Sampler is two layers of electret cloth held in mouthpiece. Breathe for 5 mins

www.youtube.com and go to our channel SAVEStudy2012.

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Results – virus overall in study

25.5 % nasal hRV +ve

11.5 % exhaled breath hRV +ve

33.3 % either nasal wash or breath hRV +ve

32.4 % breath / nasal concordant +ve for hRV

2.3 % nasal +ve for other viruses*

1.5 % breath +ve for other viruses*

56 % hRV sequenced (231 nasal, 24 breath)

*Influenza A, Influenza B, RSV, PIV1, 2, 3, hMPV

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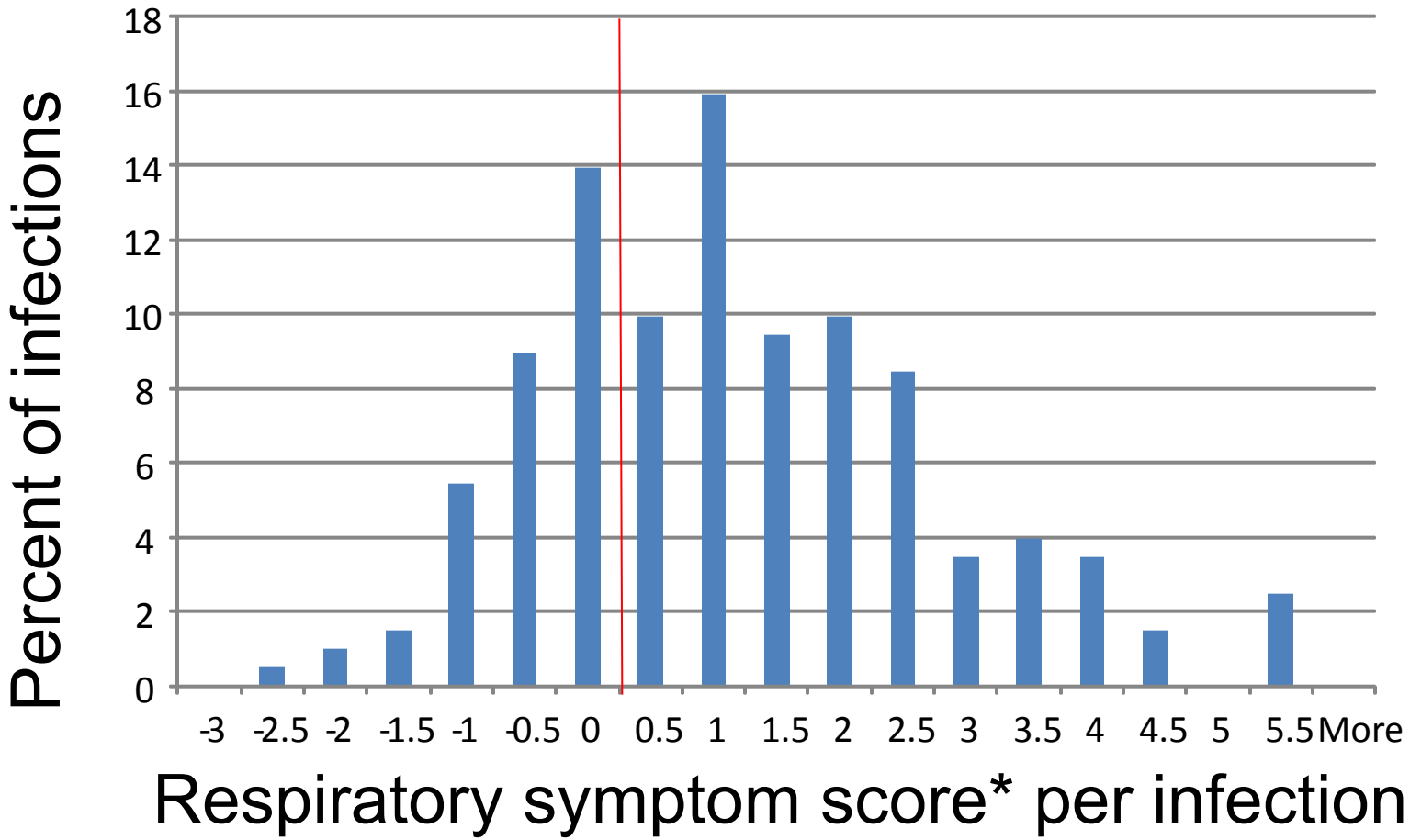
Distribution: severity of respiratory symptoms with viral infections compared to baseline



*Sum of average asthma and CCQ score, each on scale 0-3, minus average score of virus free samples for that person

Conclusions

The detection of nasal hRV was associated with a small significantly increased risk of day-to-day asthma symptoms in children.

Host, virus genotype, and environmental factors each had only a small or no effect on the relationship of viral infections to asthma symptoms.

Rhinoviruses significantly affect day-to-day respiratory symptoms of children with asthma

Euan R. Tovey, PhD,[a] Sacha Stelzer-Braid, PhD,[b,c] Brett G. Toelle, PhD,[a,d] Brian G. Oliver, PhD,[a,e] Helen K. Reddel, MBBS, PhD,[a] Christiana M. Willenborg, BSc (Hons),[b] Yvonne Belessis, MBBS, PhD,[f,g] Frances L. Garden, MBiostat,[a,h,i] Adam Jaffe, MBBS, MD,[f,g] Roxanne Strachan, BN,[g] Darryl Eyles, PhD,[j,k] William D. Rawlinson, PhD,[b,g] and Guy B. Marks, PhD[a,i]

Sydney and Brisbane, Australia

Background: Viruses are frequently associated with acute exacerbations of asthma, but the extent to which they contribute to the level of day-to-day symptom control is less clear.

Objective: We sought to explore the relationship between viral infections, host and environmental factors, and respiratory symptoms in children.

Methods: Sixty-seven asthmatic children collected samples twice weekly for an average of 10 weeks. These included nasal wash fluid and exhaled breath for PCR-based detection of viral RNA, lung function measurements, and records of medication use and asthma and respiratory symptoms in the previous 3 days. Atopy with sampling and 3 to 4 days later. No differences were found between the 3 hRV genotypes (hRV-A, hRV-B, and hRV-C) in symptom risk. A history of inhaled corticosteroid use, but not atopic status, mite allergen exposure, or vitamin D levels, modified the association between viruses and asthma symptoms.

Conclusion: The detection of nasal hRV was associated with a significantly increased risk of day-to-day asthma symptoms in children. Host, virus genotype, and environmental factors each had only a small or no effect on the relationship of viral infections to asthma symptoms. (J Allergy Clin Immunol 2015;135:663-9.)


Sometimes, even if I stand in the middle of the room, no one acknowledges me.

OUTLINE

1. What is a George Bush virus?
   - Causes your computer to keep looking for viruses of mass destruction
2. The Ronald Reagan virus?

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   - Saves your data, but forgets where it is stored

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3. The Mike Tyson Virus?
   - Quits after two bytes

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   - Saves your data, but forgets where it is stored
3. The Mike Tyson Virus?
   - Quits after two bytes
4. Jack Kevorkian Virus?
   - Deletes all old files

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OUTLINE

1. What are 10 new vaccines released in the last 10 years?

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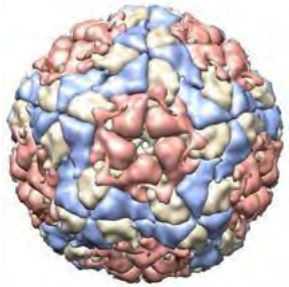
OUTLINE

1. What are 10 new viral vaccines released in the last 10 years?



Rotarix, RotaTeq (2006, 2008Rotavirus)
Gardasil (2006, HPV)
Pediarix (Diptheria/Tetanus/Pertussis/HBV/Polio)
Influenza (Every year)

CMV (2010, 2012)
VZV shingles (2006)

Others Prevnar 13 (2010, Pneumococcus), Menactra (2005,Meningococcus)

Viral Developments

- New intraspecies transmission
  - H5N1
  - Sin Nombre/hantaviruses
  - Nipah
  - Ebola
  - Arenavirus
  - nvBSE
  - HIV
  - PERV
- Transmission without clear diseases
  - Spumaretorviruses
  - Reovriuses
  - Adeno associated parvoviru ses
  - TT virus

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Viral Developments

- New diseases of old pathogens
  - hMPV
  - HRV
- Extended spread of known diseases
  - Chikungunya
  - Dengue
  - Avian influenzas
- Documentation of chronic diseases
  - HIV
  - HCV occult
  - HBV occult

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