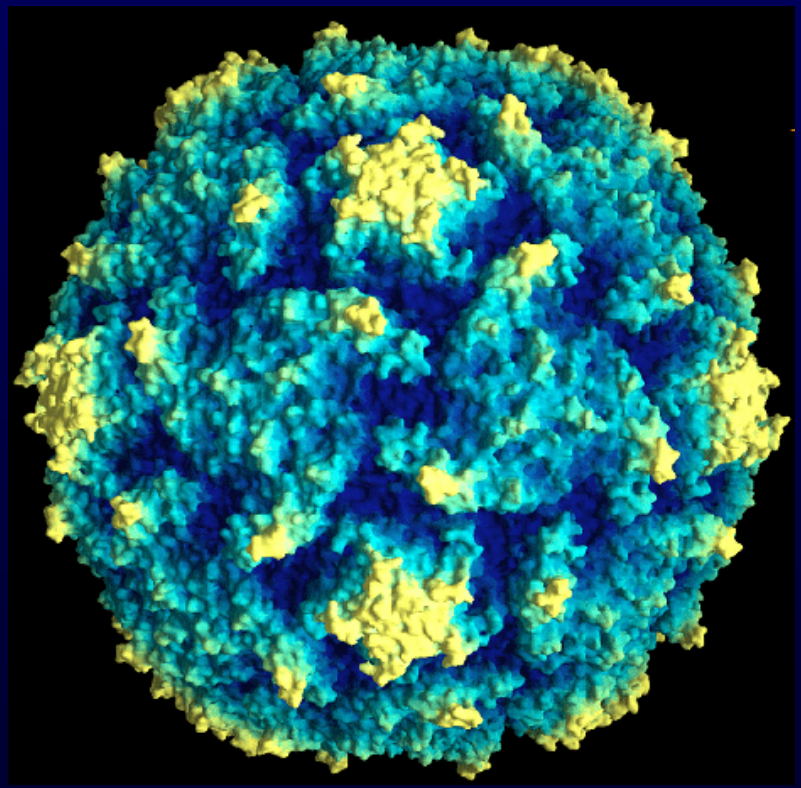
Paediatric Viral Infections: Enteroviruses and CMV



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Viruses in July 2004

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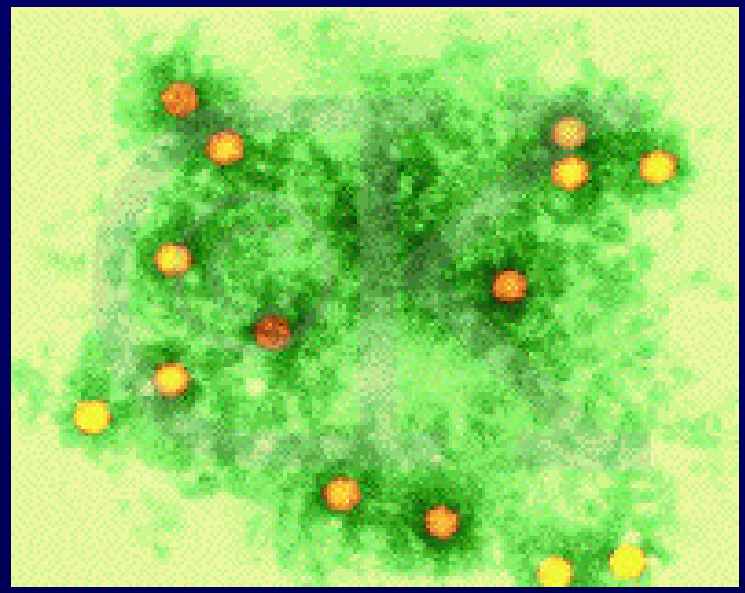
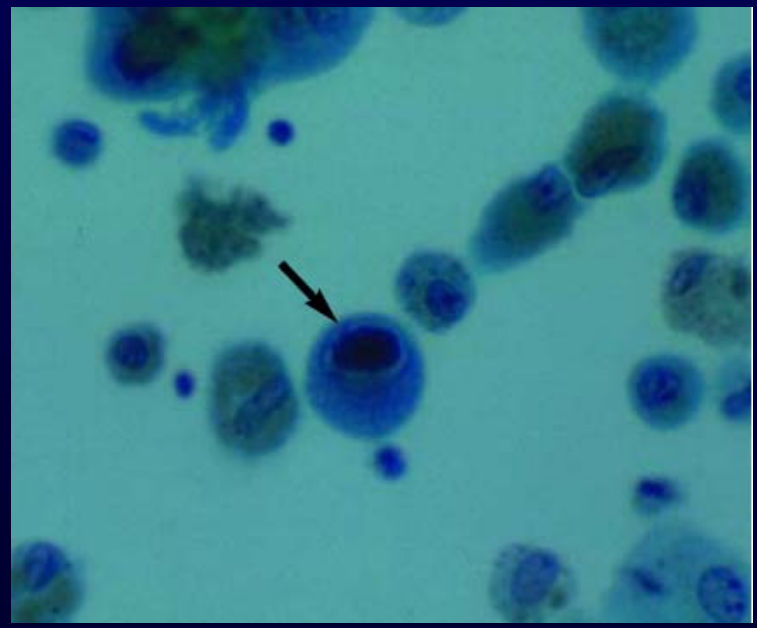
Outline

- Enteroviruses
  - Molecular virology
  - Congenital Infections
  - Paediatric Infections
  - Diagnosis
- HCMV
  - Molecular virology
  - Congenital Infections
  - Paediatric Infections
  - Diagnosis





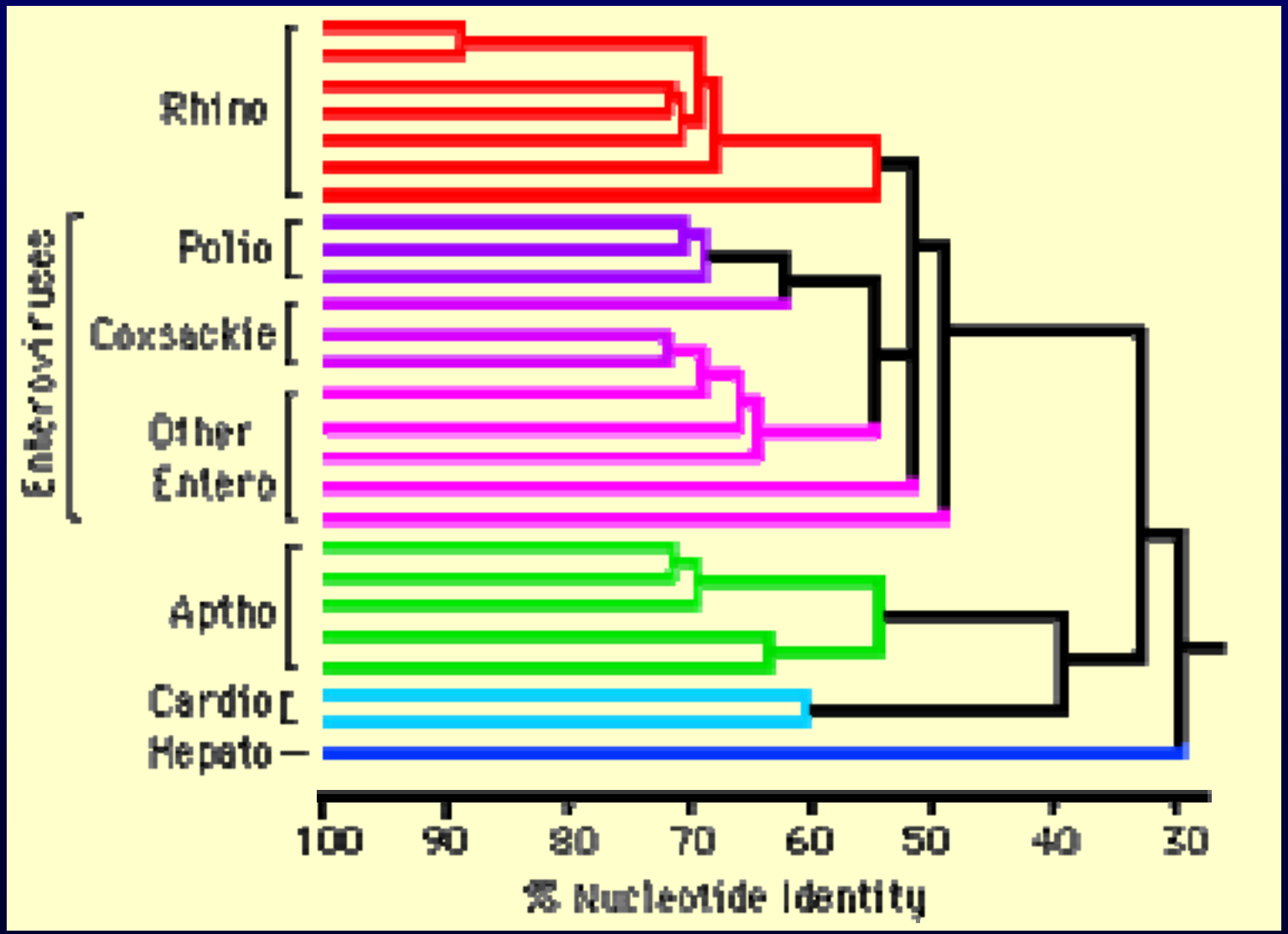
Background

- Enteroviruses are common agents in paediatric infections
- Ubiquitous
  - ~ 50 billion per year
- Transmitted by faecal oral route (infants are the “most efficient” transmitters)
  - coxsackie A21 spread by respiratory secretions
  - enterovirus 70 shed in tears, spread via fingers & fomites
- Shed in the upper respiratory tract for 1-3 weeks & in faeces for up to 8 weeks after primary infection
- Cause a wide spectrum of common and uncommon illnesses
  - Often asymptomatic or mild illness
  - Severe infection & death

Picornaviruses

- Diverse family > 200 serotypes
- ‘Oldest' known viruses
  - records from Egypt ~ 1400 BC
- FMDV was one of the first viruses to be recognised - Loeffler and Frosch 1898
- Polio was first recognised as a viral disease by Landsteiner & Popper in 1909

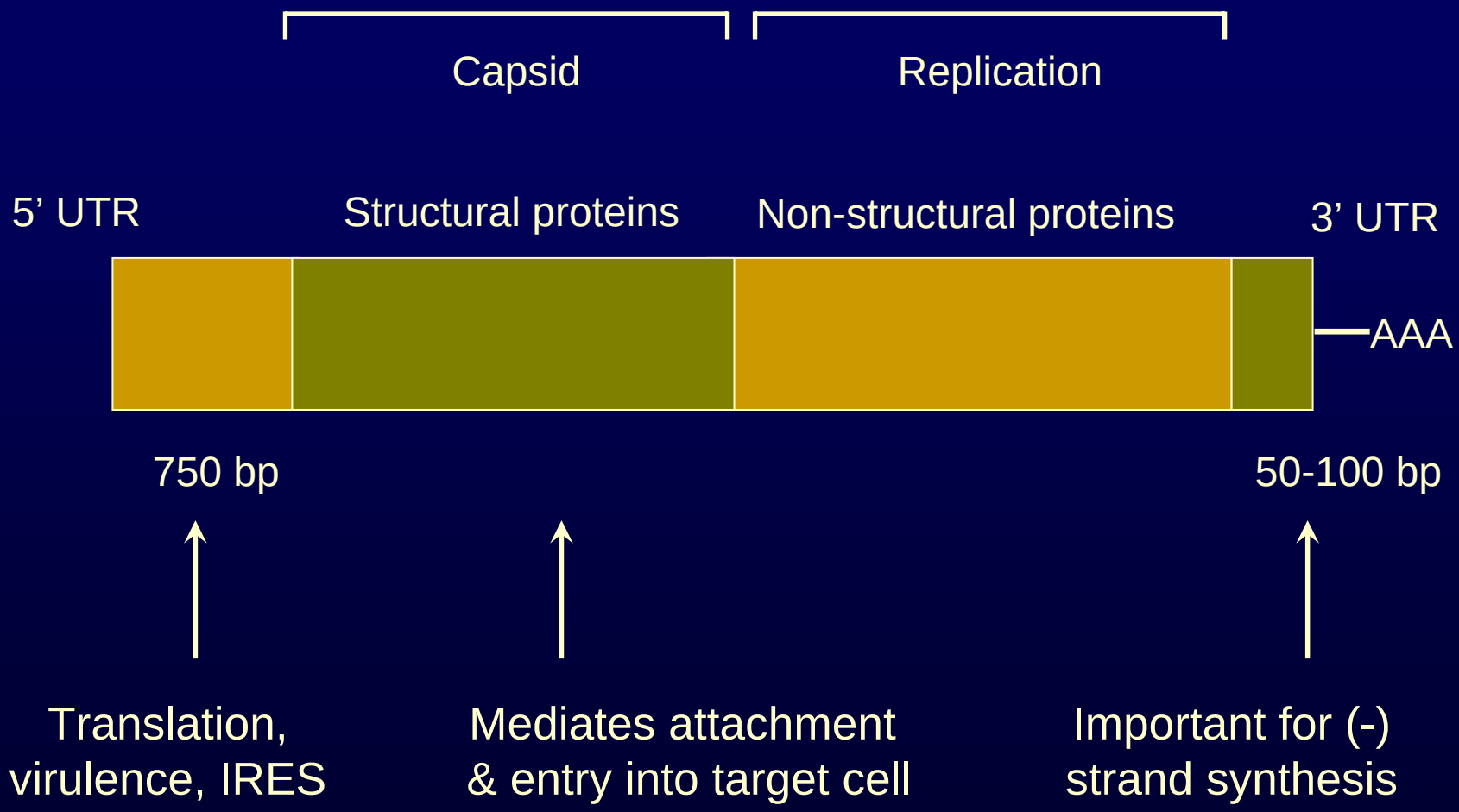
Picornavirus phylogeny



Enterovirus structure

- Small icosahedral, non-enveloped viruses
- 27 –30 nm, 7500 - 8500 nucleotides long
- Simple viral capsid & single positive strand RNA
  - capsid is composed of 60 densely packed copies of capsid proteins VP1, VP2, VP3 & VP4
- Antigenic diversity is due to capsid protein variation (VP1 – VP3)
- VP4 functions as an anchor to the viral capsid
  - destabilisation of VP4 results in viral uncoating
- Replication cycle is rapid, usually 8 hours
  - Occurs in cytoplasm

Enterovirus Genome Structure



Genetic Subtyping of EVs

- "Classical" subtyping was based on:
  - disease caused in suckling mice
    - CAVs vs CBVs
  - particle density & pH sensitivity
  - **E**nteric **C**ytopathic **H**uman **O**rphan viruses
- After 1969, new EVs were given numbers
  - EV 68: pneumonia
  - EV 70: acute haemorrhagic conjunctivits
  - EV 71: meningitis & rhomobencephalitis

Current Enterovirus nomenclature

- Human enterovirus A (HEV A):
  - CV -A2, -A3, -A4, -A5, -A6, -A7, -A8, -A10, -A12, -A14, -A16, EV-71
- Human enterovirus B (HEV B):
  - CV -B1 to -B6, CV-A9, Echovirus (E) -1 to -9, E-11 to -21, E-24 to -27, E-29 to -33, EV-69
- Human enterovirus C (HEV C):
  - CV -A1, -A11, -A13, -A15, -A17 to -A22, -A24
- Human enterovirus D (HEV D):
  - Enterovirus (EV) -68, -70

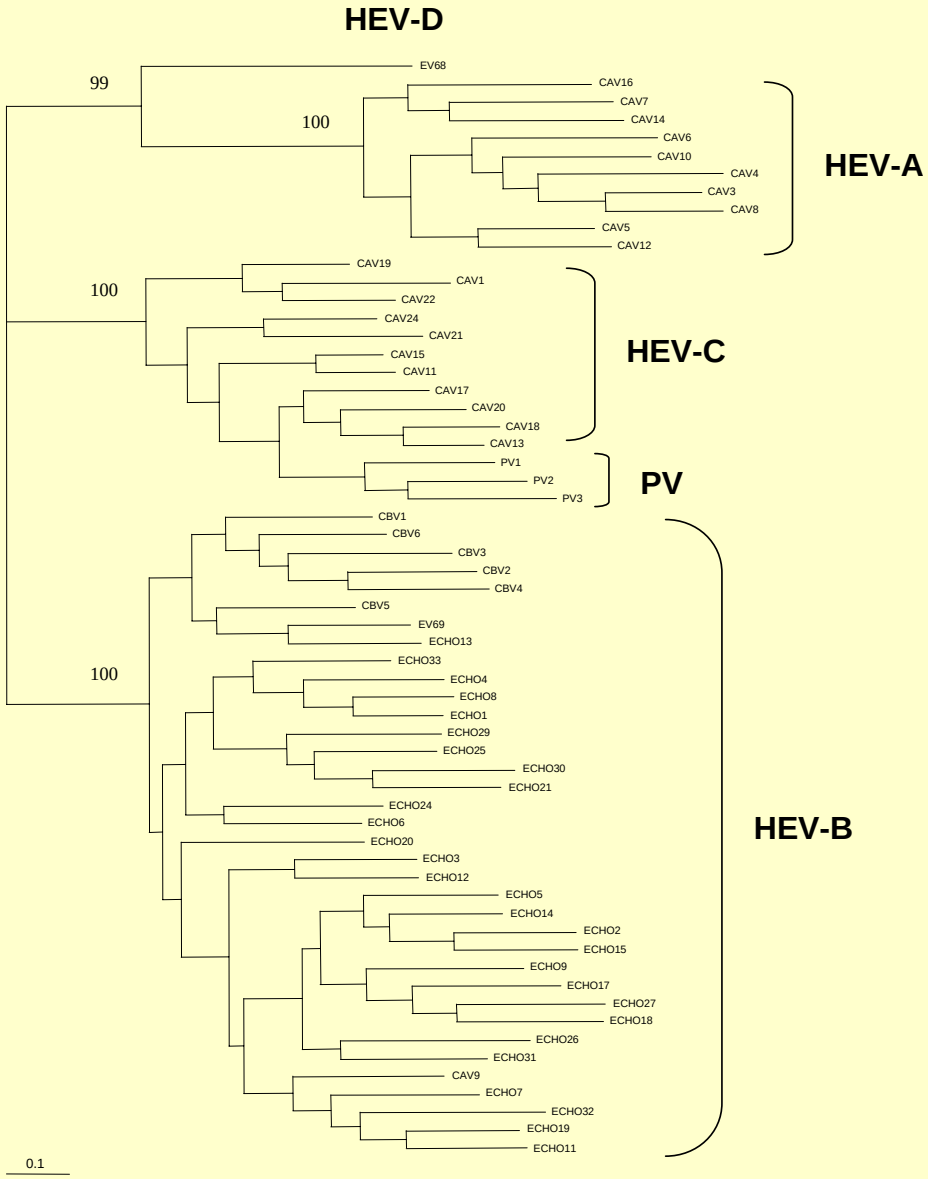
Genus Enterovirus cont.

- Poliovirus
  - although close to HEV C, separate species due to unique clinical features and receptor usage
- Bovine enterovirus
- Porcine enterovirus A
- Porcine enterovirus B
- Unassigned enteroviruses
  - mostly Simian EVs
- *New classification (A-D) based on 3' & 5' UTR*

Phylogeny of Enteroviruses

- VP1 CAPSID: 4 main groups
  - Cluster A: CAVs, EV 71
  - Cluster B: CAV 6, CBV 1, E26, EV 69
  - Cluster C: CAV 19, CAV 24, PV 1
  - Cluster D: EV 68, EV 70
- Virus evolution - EVs probably all derived from a single virus
  - capsid proteins are targets of host immune surveillance, so allow EVs to broaden their “niche”
  - EV diversity is reflected in the variety of cell surface molecules they recognise as they enter host cells (at least 6 membrane proteins interact with EVs)

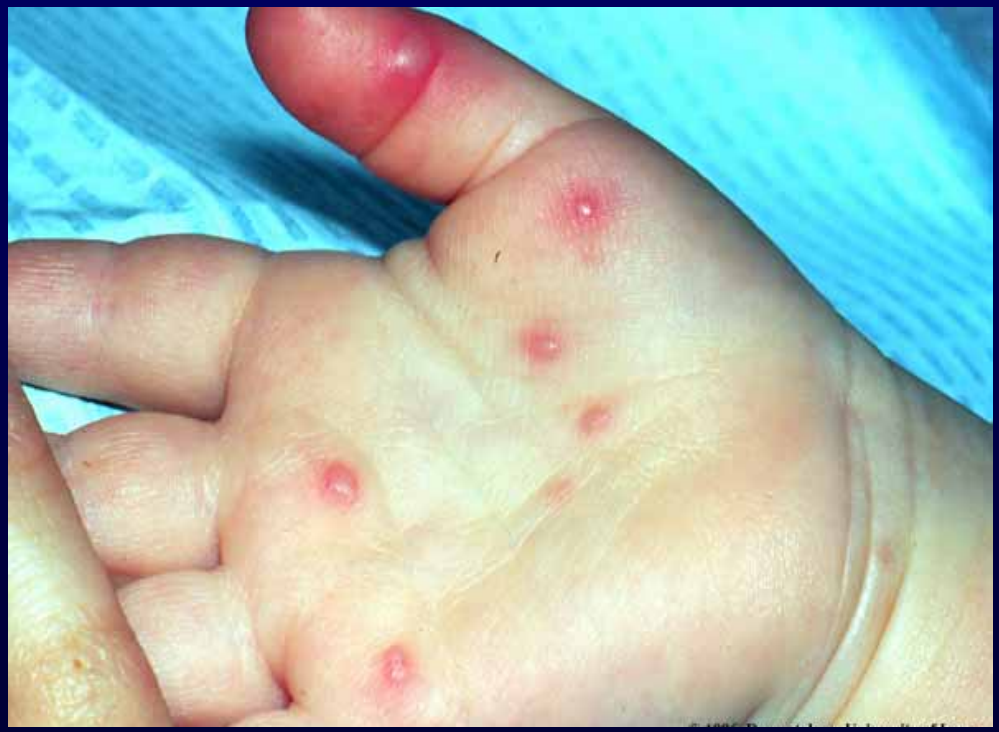
Phylogenetic analysis of the VP1 gene

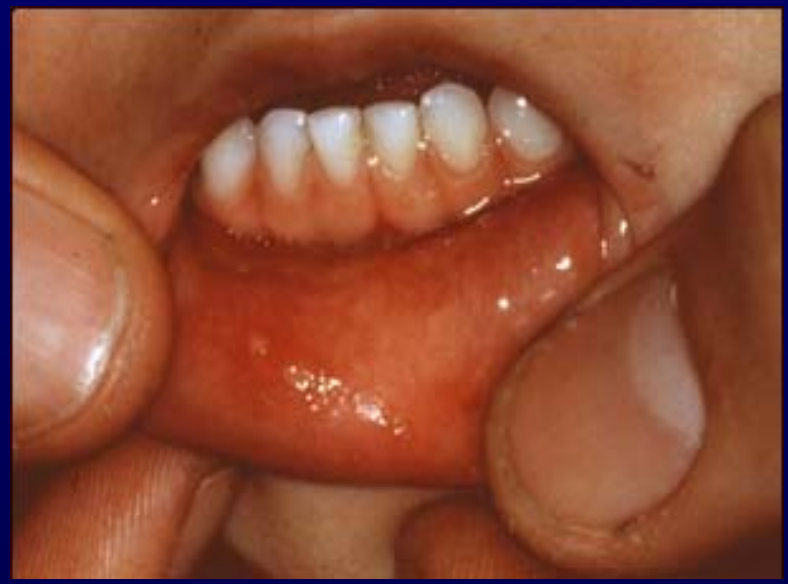


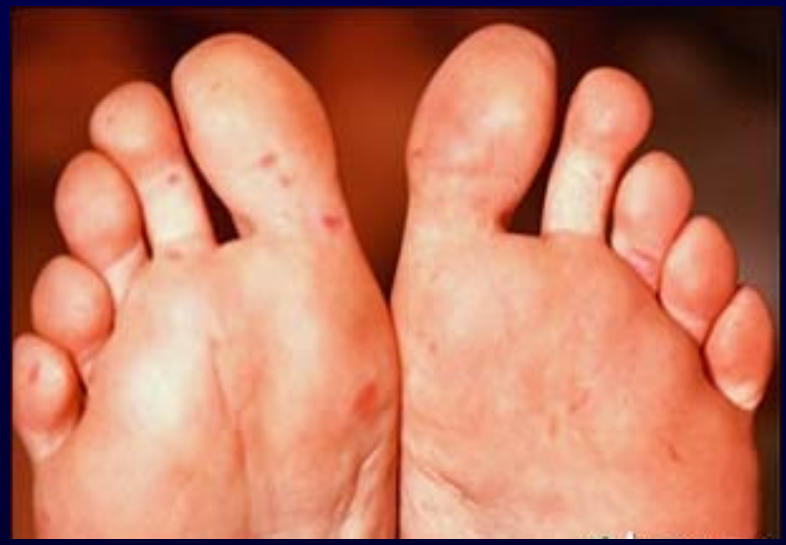
Enterovirus Infections

- Exanthema – Hand foot & mouth disease
- Non-focal acute febrile illness
  - ~ 50 – 60% of infants < 3 mths
- Respiratory illness
- Gastroenteritis
- Encephalitis / Meningitis
- Myocarditis
- SIDS
- Acute haemorrhagic conjunctivitis (EV 70/CAV 24)
- Pancreatitis / Type 1 diabetes

Hand, foot and mouth disease







Congenital EV infections

- Case reports
- Clinical features
  - Cerebral palsy
  - Diabetes
  - Hepatitis
  - Jaundice
  - Thrombocytopaenia
  - Generalised infection

Neonatal Enterovirus infection

- Represents a significant proportion of “PUO”
- Presenting features include
  - asymptomatic/mild infection
  - poor feeding, lethargy, convulsions, tremor, hypotonia, diarrhoea
- Clinical manifestations include
  - hepatic necrosis, meningoencephalitis, myocarditis, fever, rash, sepsis, respiratory illness/pneumonia
- The absence of maternal symptoms does not preclude infection in the neonate
- Early onset < 6 days
  - usually due to maternal transmission
- Late onset $\geq$ 7 days
  - postnatal maternal/ family member /nosocomial transmission

Neonatal Enterovirus infection

- Investigations
  - Infant (and maternal) samples
  - Culture, PCR, serology
- Treatment
  - IVIG
  - Polio vaccine
  - Pleconaril – limited experience

Respiratory illness

- Jartti et al, Emerg Infect Dis June 2004
- 2-year prospective study in Finland
  - 293 hospitalized children
  - NPA: enteroviruses (25%), rhinovirus (24%), non-typable rhino/enterovirus (16%) were found most frequently; RSV (27%)
  - In older children, respiratory picornaviruses dominated (65% of children ages 1-2 years and 82% of children ages > or =3 years)

Myopericarditis

- CVBs are cardiomyotropic
- ~ 33 – 50% of sporadic cases
- Most cases in epidemics
- ~ 5% fatality rate
- viral replication in the myocardium peaks within 3-7 days & persists for 7-10 days in immunocompetent hosts,
  - longer in the immunocompromise d
- adolescents / young adults at highest risk;
  - males twice the risk of females

EV 71 epidemics

- South East Asia & Australia
- 1997 - 1999
- Hand, foot, and mouth disease (common)
- Severe disease, including pulmonary oedema & invasive CNS disease
  - aseptic meningitis, Guillain-Barre´ syndrome, acute transverse myelitis, acute cerebellar ataxia, opso-myoclonus syndrome, BIH
    - McMinn et al, Clin Inf Dis 2000
  - Survival related to Rx with ?pleconaril, steroids, IVIG, vigorous resuscitation, afterload reduction
    - Nolan et al J Neurology 2002

Clinical features – EV 71 cases

Patient	Greatest deficit	Acute MRI lesions (day)	Late MRI lesions	Long term deficit (17-86 months)
1	**Opthalmoplegia,** facial weakness, bulbar dysfunction, no resp effort, AFP all 4 limbs	whole brainstem cervical*	whole brainstem whole spine	**Died 9** weeks into illness, only grimace, weak movement of eyes & R hand
2	Facial weakness, bulbar dysfunction, poor resp effort, AFP all 4 limbs, UL L>R	medulla cervical	L cervical (C4 only)*	Normal other than weak L shoulder & elbow
3	**Opthalmoplegia,** facial weakness, bulbar dysfunction, poor resp effort, AFP UL R>L, LLs strong	pons medulla cerebellum cervical thoracic R>L	ND	Normal other than weak R shoulder & R elbow flexion
4	**Opthalmoplegia,** facial weakness, bulbar dysfunction, no resp effort, myoclonus, urinary retention, AFP all 4 limbs	pons medulla cervial thoracic	medulla whole spine	Weak gag, some resp effort but ventilator dependent, L UL weakness, other limbs normal & walking independently
5	**Opthalmoplegia,** facial weakness, bulbar dysfunction, no resp effort, myoclonus, urinary retention, AFP all 4 limbs	pons medulla whole spine	whole brainstem whole spine	No gag, some independent resp effort but ventilator dependent, functional ULs (L weaker than R), severe weakness LLs
6	**Opthalmoplegia**, facial weakness, bulbar dysfunction, no resp effort, urinary retention, AFP all 4 limbs	pons medulla cervical	pons medulla whole spine	Fully ventilator dependent, only movement is head nod, facial expression and very limited R hand function
7	**Opthalmoplegia**, facial weakness, bulbar dysfunction, no resp effort, AFP UL, some LL movement	whole brainstem cerebellum cervical*	pons medulla	Normal strength, diaphragm pacing allows independent daytime ventilation, nocturnal ventilation still required

EV 71 Transverse Myelitis

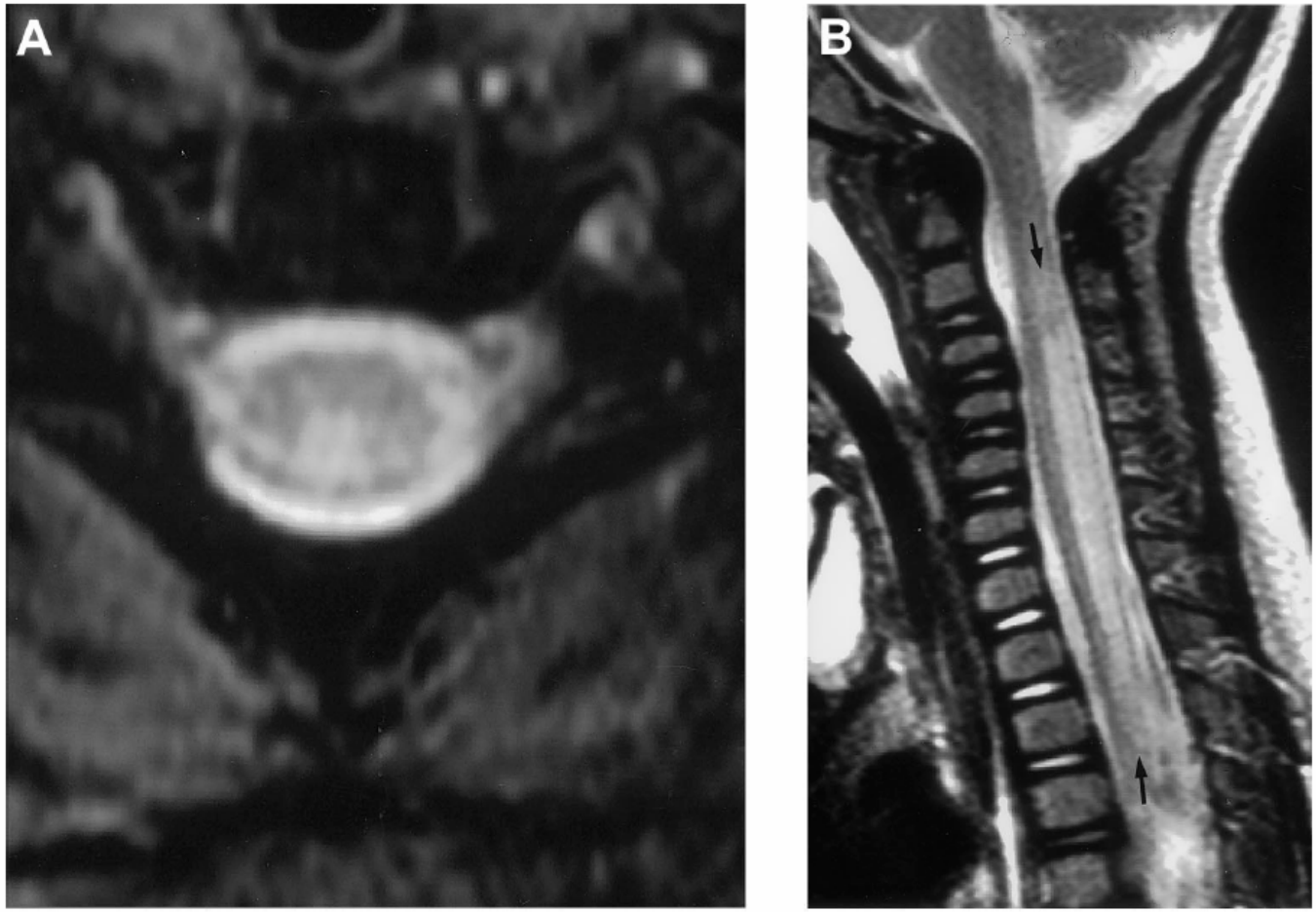
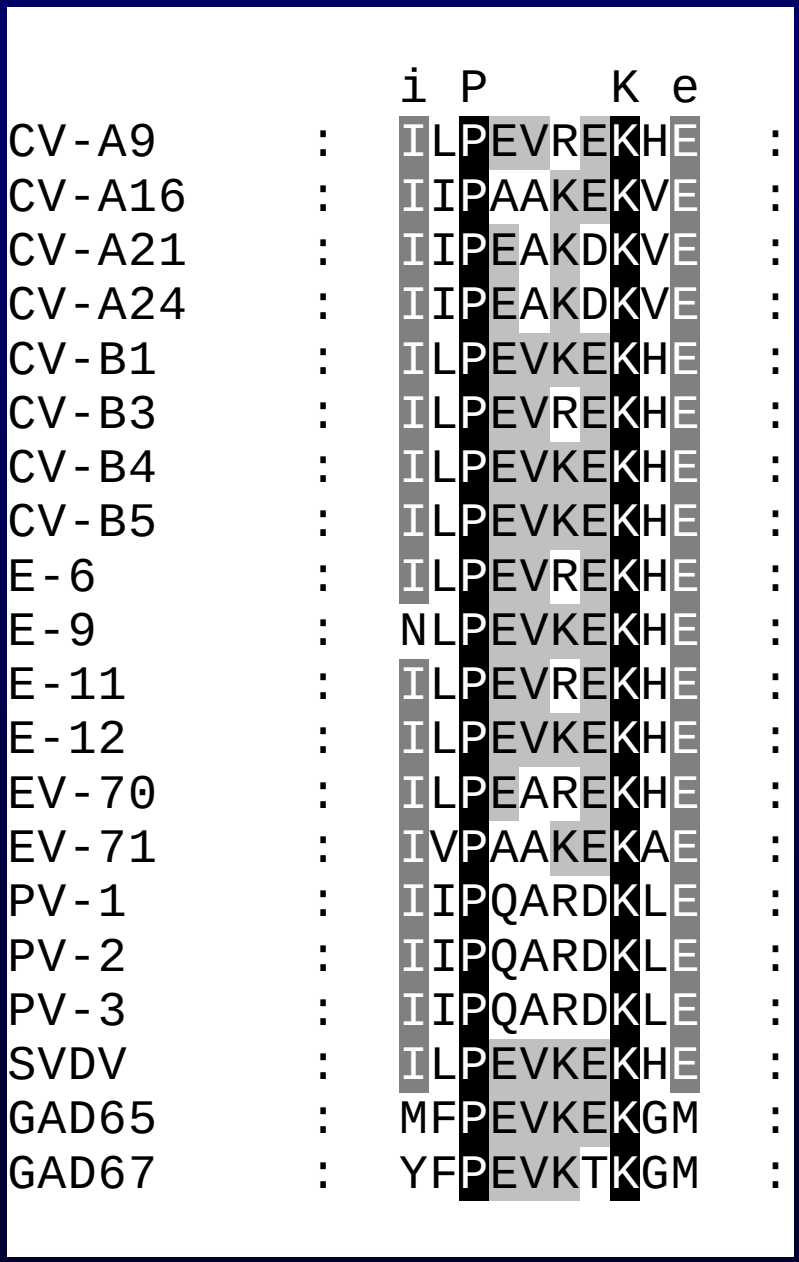


Figure 1. MRI of a 9-month-old female infant (patient 2) with enterovirus 71–associated neurological disease. *A,* axial gradient-echo T_2-weighted MRI done 3 days after the onset of acute transverse myelitis, showing a high signal lesion centered in the dorsal column white matter of the cervical cord. *B,* midsagittal turbo spin-echo T_2-weighted MRI scan done during the same examination as in *A,* showing a lesion from C2 to T2 *(arrows)* with mild cord expansion.

Type 1 diabetes and EVs

- Enteroviral association with type 1 diabetes is well known, but not well understood
- Mechanism of involvement in diabetes pathogenesis is unclear
  - molecular mimicry
  - innocent bystander
  - direct infection
- Early studies suggested predominance of Coxsackieviruses (B4)



Amino acid sequence homology between GAD65 and Enterovirus 2C protein

The evidence

- When:
  - Enteroviruses at diagnosis
  - Prospective studies of children at risk
  - In utero infection
  - Temporal association with Ab conversion
- How:
  - Serologic studies
  - Studies of pancreata and cultured islets
  - Animal studies
  - Detection of RNA in serum, buffy coat, stool

EV and Diabetes Study

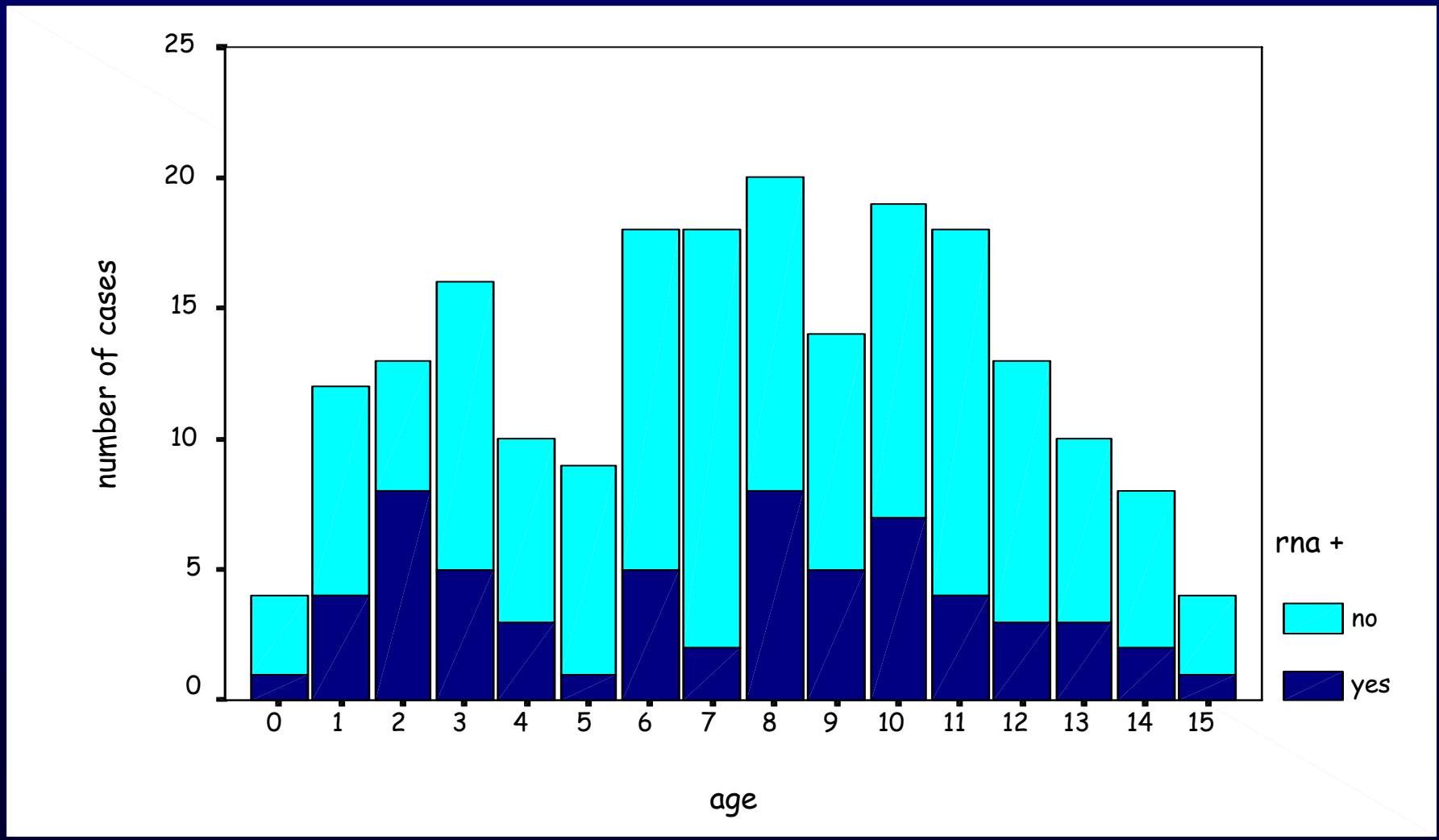
- Case-control study
- 206 children from Western Sydney diagnosed between April 1997 - Sept 1999
- 160 age matched healthy controls
- Plasma & stools samples collected for RT-PCR from diabetic & control subjects
- Serum and DNA in diabetic children
  - HLA typing & diabetes –associated autoantibody analysis
  - ELISA for heterotypical IgM, IgA and IgG

Results

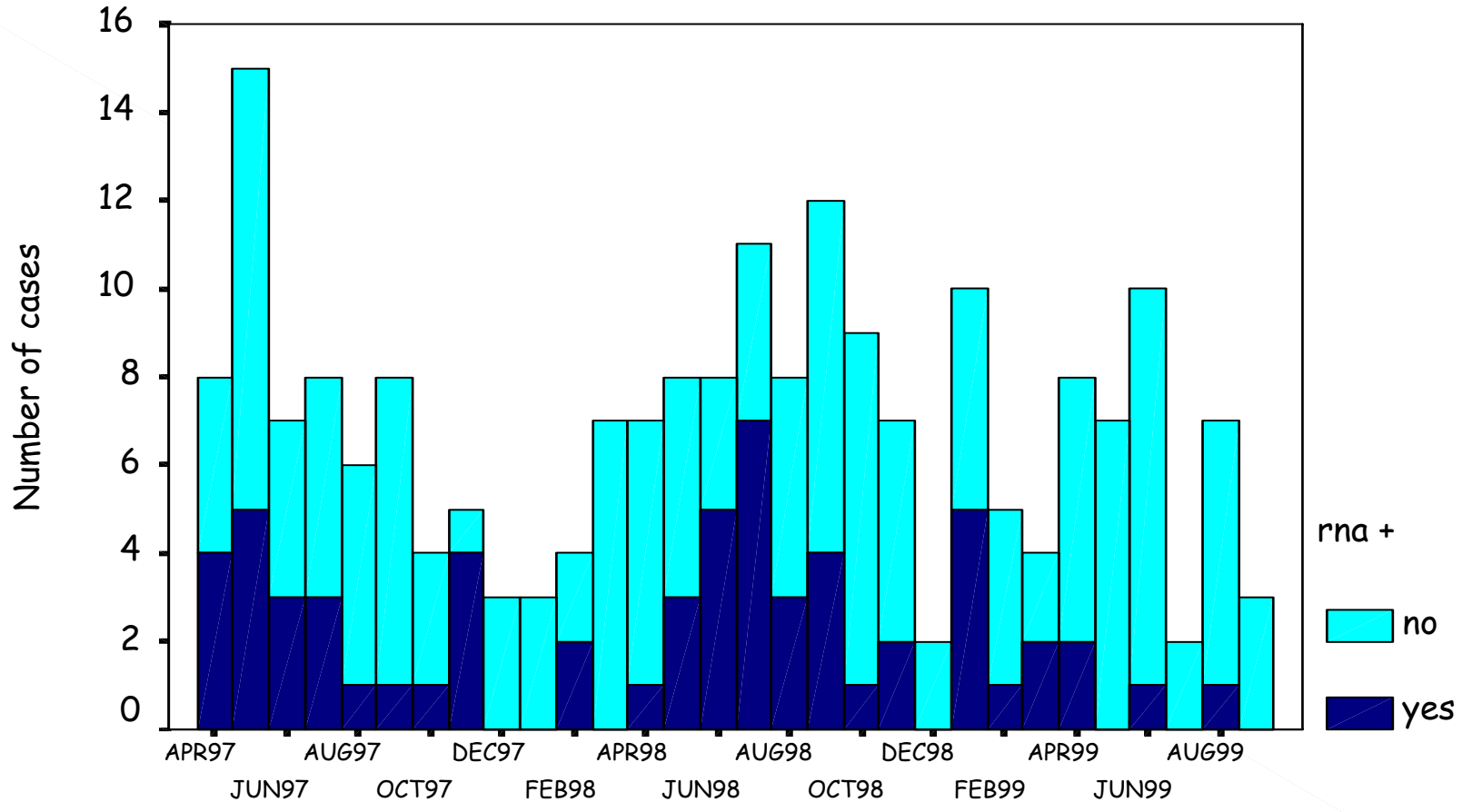
	Diabetes	Control	p
PCR +	62/206 (30%)	6/160 (4%)	<0.001
Stool	26/110 (24%)	4/25 (16%)	NS
Plasma	58/206 (28%)	3/160 (2%)	<0.001

Craig et al, J Inf Dis 2003

Age distribution



Seasonal pattern of infection



Multiple enterovirus subtypes

Enterovirus 71	17	(28%)
Coxsackie B1	14	(23%)
Coxsackie B3	7	(11%)
ECHO 30	4	(7%)
CAVs	3	(5%)
Other	11	(18%)
Not typable	5	(8%)
Polio (Sabin) - excluded	2	

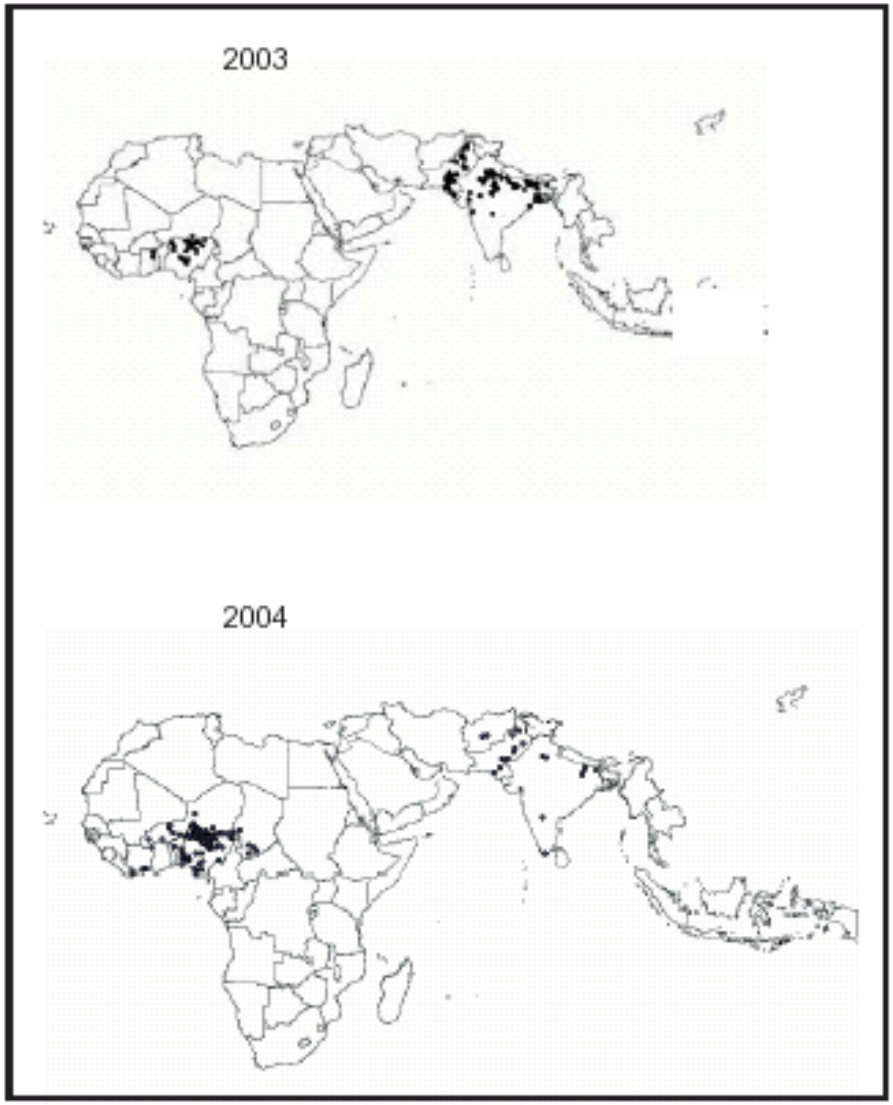
Further analysis

- Negative association with genetic predisposition (DR3 or DQB1*02) implies a “viral subgroup” of type 1 diabetes
- Children with high C-peptide at diagnosis (>90th percentile) were less likely to be enterovirus RNA positive
- Severe DKA at diagnosis (pH <7.1) was significantly associated with enterovirus RNA positivity
- No association with autoimmune markers
- No association with gender, BMI, history of infection

Polio

- In 1988, the World Health Assembly resolved to eradicate poliomyelitis globally
- Countries with endemic polio decreased from 125 in 1988 to six in 2003
- But in 2003, 10 countries reported poliovirus importations,
  - West and Central Africa (8), Southern Africa (Botswana), and Middle East (Lebanon)

FIGURE. Number* and location of virus-confirmed poliomyelitis cases, January–April 2003 and January–April 2004†



* A total of 135 during January–April 2003 and 185 during January–April 2004.
† As of May 18, 2004.

Acute flaccid paralysis - worldwide

TABLE. Acute flaccid paralysis (AFP) and poliomyelitis cases, by World Health Organization region and country, 2003 and 2004*

Region/Country†	No. reported AFP cases		Nonpolio AFP rate§		% persons with AFP with adequate specimens¶		Virus-confirmed cases			
							Total		January–April	
	2003	2004	2003	2004	2003	2004	2003	2004	2003	2004
African	8,184	2,745	2.6	2.7	88	91	446	162	34	162
Nigeria	3,318	1,425	6.0	7.9	91	91	355	133	32	133
Niger	175	80	2.4	3.6	79	88	40	12	1	12
Eastern Mediterranean	5,294	1,798	2.4	2.3	90	90	113	15	24	15
Pakistan	2,270	742	3.0	2.8	90	90	103	12	23	12
Afghanistan	599	212	3.9	3.9	88	91	8	2	0	2
Egypt	608	268	2.5	2.7	93	94	1	1	0	1
South-East Asian	11,305	3,360	1.9	1.1	83	85	225	8	77	8
India	8,524	2,543	2.0	1.1	81	84	225	8	77	8
American	2,229	488	1.3	0.8	80	—	0	0	0	0
European	1,639	491	1.2	1.0	82	81	0	0	0	0
Western Pacific	6,397	1,313	1.4	0.9	88	85	0	0	0	0
Worldwide	35,048	10,195	1.9	1.5	86	87	784	185	135	185

* 2004 data are cases reported during January–April, as of May 18, 2004.
† Data presented only from countries with indigenous polio during 2003. Values do not add to regional and global totals.
§ Per 100,000 children aged <15 years; annualized for 2004.
¶ Two stool specimens collected at an interval of at least 24 hours within 14 days of paralysis onset and adequately shipped to the laboratory.

Diagnosis of Enterovirus Infection

- Viral culture
- Serology
  - Complement Fixation
  - Neutralisation
  - ELISA
- PCR

Viral culture

- Traditional, “gold standard”
- Relatively sensitive, and yields an isolate that can be further serotyped for clinical or epidemiologic purposes
- BUT
  - takes 3 - 7 days
  - expensive
  - requires cell lines
  - some types eg CAVs difficult to culture

PCR

- Rapid result ~ hours
- Increased sensitivity compared with culture (some studies > 90%)
- Detects multiple subtypes in one assay
- Use of specific primers (eg VP1) or sequencing allows genotyping of isolates
- Can improve patient management
  - decreased hospital length of stay for children with enteroviral meningitis

ELISA

- Low sensitivity
- High specificity for IgM
- Useful for retrospective diagnosis
- Cheap, large number of specimens can be processed
- Depends on background immunity of population

PCR vs ELISA for EVs

ELISA	No. ELISA positive/ No. PCR-Positive	Sensitivity (%)	No. ELISA positive/ No. PCR negative	%	P-value	Specificity (%)
IgA	20/68	**29**	70/297	24	0.3	76
IgM	19/68	**28**	11/297	4	<0.001	**96**
IgG	37/68	**54**	102/297	34	0.002	66
IgM or IgG	48/68	71	107/297	36	<0.001	64
IgA or IgG	42/68	62	137/297	46	0.02	54
IgA or IgM	32/68	47	78/297	26	0.001	74
IgA, IgM or IgG	50/68	**74**	141/297	47	<0.001	**53**

Craig et al, J Clin Micro 2003


Reynolds
PEPSI
PEPSI
"You're fired, Jack. The lab results just came back, and you tested positive for Coke."

Questions ?

Human Cytomegalovirus (HCMV)

- DNA virus – *Herpesviridae*
  - Large, enveloped viruses
  - Properties of latency and reactivation
- Genome consists of DS DNA ~ 200 kilobase pairs
  - codes for more than 200 ORFs
- Subtyping based on variation in glycoprotein B (gB)
  - Correlates with viral tropism *in vivo*
  - Variation in gB may influence CMV virulence
- Ubiquitous
  - adult seropositivity rate ~ 60 - 100%
  - Aust Red Cross seropositivity rates
    - ~ 40% at 20 yrs to 70% at 50 yrs
- Transmission
  - breast feeding, sexual contact, vertical transmission, spread from children, transplanted organs

Human CMV infection

- Mainly asymptomatic in healthy individuals
  - 10% have mononucleosis-like illness
    - malaise, persistent fever, myalgia, cervical lymphadenopathy
    - less common pneumonia, hepatitis
    - laboratory findings include atypical lymphocytes, mild thrombocytopenia and elevated liver enzymes
  - infection is self-limited
    - viral excretion may be prolonged
    - CMV persists throughout life
- Severe infection in immunocompromised host, fetuses and neonates

Reactivation vs reinfection

- Recurrent infection (intermittent excretion of virus from single or multiple sites)
  - Reactivation of an endogenous virus (more common)
  - Exposure to a new virus strain from an endogenous source (less common)
  - Mixed infection may also occur
- Reasons for recurrence
  - Low grade chronic infection following primary infection, with intermittent detection of virus due to low copy numbers
  - Reactivation of latent virus in response to stimuli eg pregnancy

CMV diagnosis

- Viral culture – MRC5
  - > 2 weeks
- Serology
  - IgM, IgG
  - IgM Avidity
- PCR
  - Qualitative & quantitative
  - *In situ*
  - Multiplex

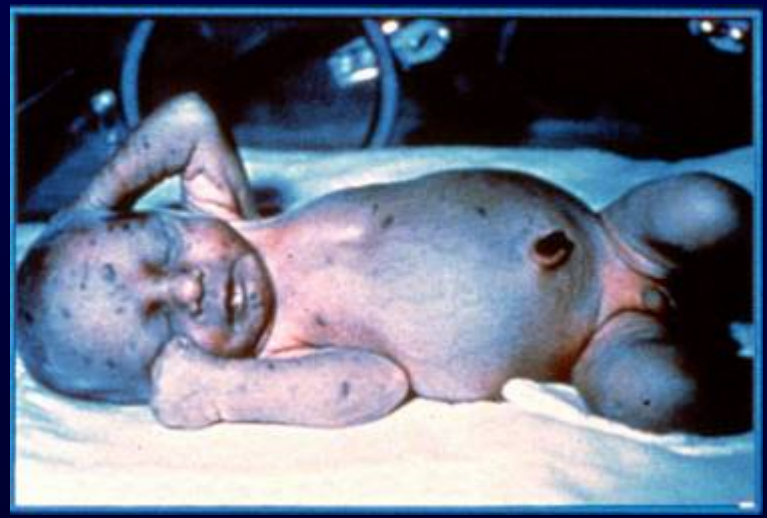
Table 2 Tests routinely used to detect infection with cytomegalovirus

Test	Principal uses	Potential problems	Specimens
Antigen detection:			
Virus culture	Virus detection Virus for further study using PCR, genotyping, antiviral susceptibility testing	Long time to result (3–4 weeks) Expensive set-up costs for virus culture laboratory Confusion of cytopathic effect (CPE) with adenovirus CPE Specimen contamination Culture positivity with reactivation ± disease	Urine, blood, tissue
Direct immunofluorescence	Rapid detection of virus – results available in 48 h	Culture positivity with reactivation ± disease Limited culture is still necessary	Blood, urine
Nucleic acid testing	Rapid detection of virus Quantification of viral load	Cost of individual test high Contamination resulting in false positives	Urine, blood, CSF, Tissue
	Virus strain typing	Various techniques (PCR, bDNA, NASBA, TMA) Acceptable for diagnosis if correlated with active CMV infection	
Histopathology	Definitive demonstration of tissue damage	Need for a clinical procedure False negative rate high	Tissue
In situ hybridization	Definitive demonstration of CMV		Tissue
Antibody detection:			
IgG-EIA	Show previous infection Show recent infection with seroconversion Avidity shows acute infection	Seroconversion takes 2–3 weeks, needs two samples False seroconversion with administration of blood products or immunoglobulin (Ig) Detect recent infection (avidity < 60%)	Serum
IgM-EIA		Seropositivity for 2 years post acute infection in 5% Cross-reactivity with EBV (rare) Sensitivity of single cord blood IgM 70–80% Antigenic heterogeneity in clinical isolates[18]	Serum
Complement fixation	Demonstration of rising titre	False negative in 2–5%	Serum

Trincado & Rawlinson, JPCH 2001

Congenital CMV

- Most common cause of congenital infection
  - 0.3 - 2.4% of neonates are infected with CMV
  - Increased rate if premature (4.8% < 34 weeks)
    - Panhani et al, Scand J Infect Dis 1994
  - Higher in populations of lower SES
  - ~10% symptomatic
  - 10 - 30% mortality
- Primary infection or reactivation



Epidemiology

- 1 - 3% of pregnant women develop primary CMV infection
  - 30 – 40% of infants are congenitally infected
  - Of these
    - 10 – 15% symptomatic
      - 20 – 30% mortality (DIC, hepatic dysfunction, bacterial superinfection)
      - 70 – 80 % of symptomatic infants will develop complications in first few years of life
    - 5% -10% infected but asymptomatic infants at birth will develop later sequelae
- In women who have CMV infection at least 6 months prior to conception
  - ~1% infants are congenitally infected
  - Most asymptomatic

Clinical features - congenital CMV

- Asymptomatic
  - reactivation >>> primary infection *
- Microcephaly*
- Thrombocytopaenia*, petichiae
- IUGR*, prematurity
- Hepatosplenomegaly* / jaundice
- Sensorineural hearing impairment
  - 40% severe – impaired communication/learning
  - 80% detected > 1 year old
- Cerebral Palsy / Mental retardation
- Chorioretinitis

* Most common findings: Boppana et al, Ped Inf Dis J 1992

Symptomatic vs asymptomatic

- More severe or atypical manifestations & higher mortality in preterm infants (NB small nos)
  - Yamamoto et al, Paed Inf Dis J 2001
  - Perlman et al, Ann Neurol 1992
- Earlier studies suggested symptomatic congenital CMV infection usually associated with primary maternal infection
  - Stagno et al, N Engl J Med 1982
  - Fowler et al, N Engl J Med 1992
- Recent studies show symptomatic congenital infection in highly seropositive populations
  - Ahlfors et al, Scand J Infect Dis 1999
  - Boppana et al, Pediatrics 1999
  - Yamamoto et al, Paed Inf Dis J 2001

Diagnosis of congenital infection

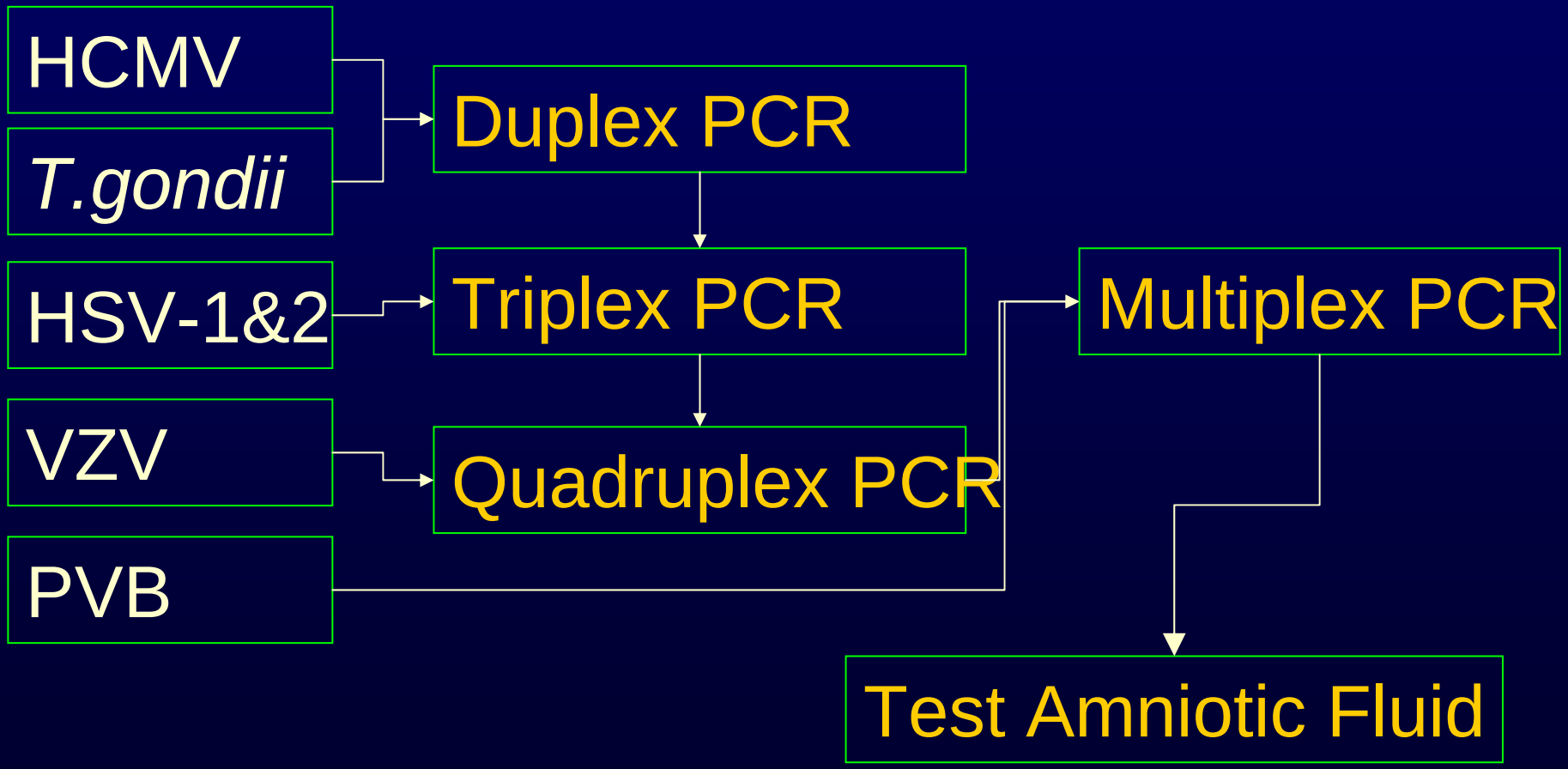
- Amniotic fluid testing
  - Multiplex PCR
- Cordocentesis
  - fetal blood
- Urine culture
- Serology

AF testing for CMV

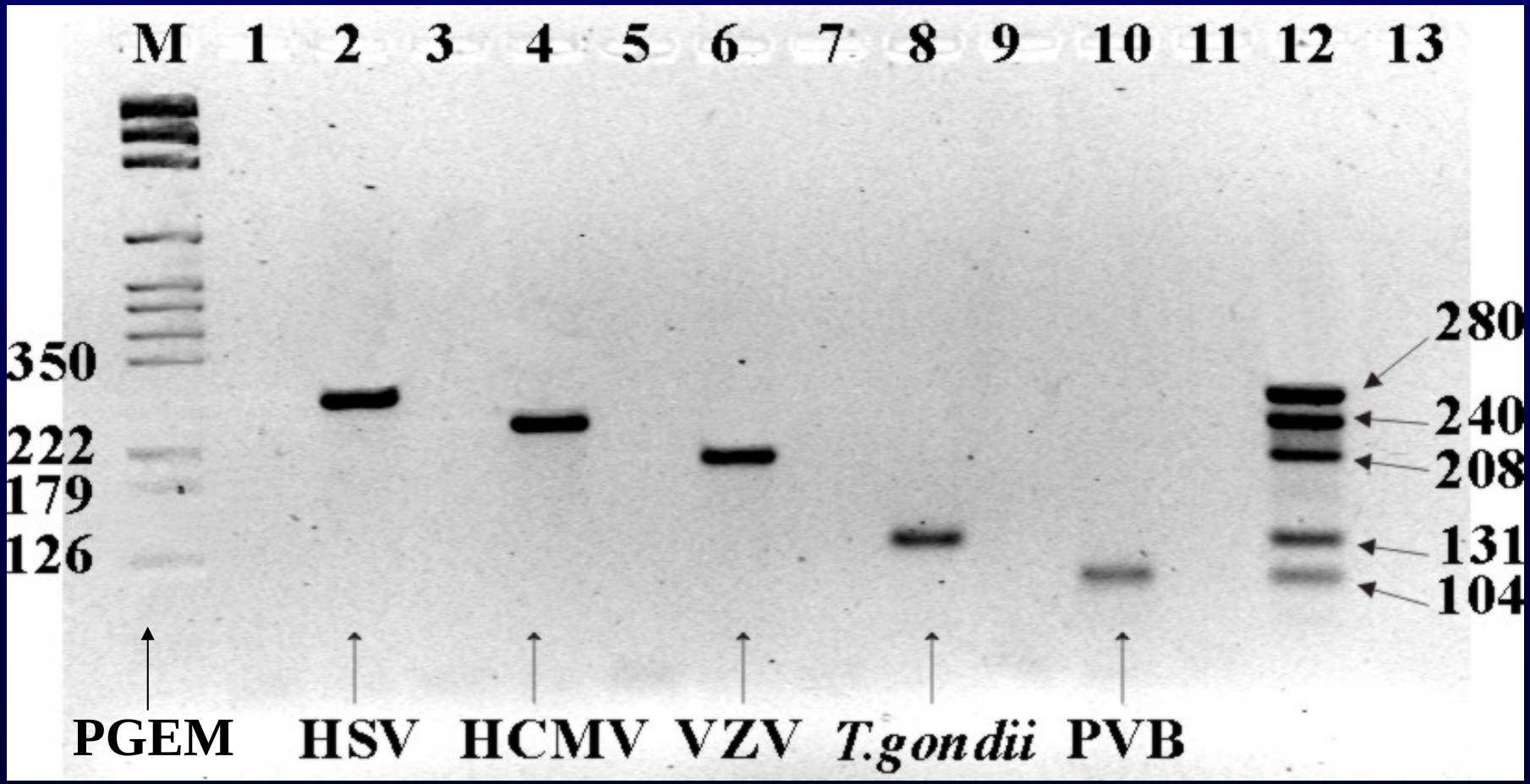
	Author	Time of collection	Total cases	PCR +ve Results		Post natal isolation
				CMV	VZV	
R	McLean el al1995	1994	277	0	ND	ND
T	Mouly et al. 1997	1989-94	107	ND	8.4%	3.8%
T	Liesnard et al 2000	1985-98	237	29%	ND	24%
T	Lipitz et al. 1997	1992-95	66	35%	ND	35%

R = Random trial, T = Targeted trial

Multiplex PCR Development



Multiplex PCR



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