

HUMAN RHINOVIRUSES

Sneeze, wheeze and the ABCs

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Take home messages

The study of HRVs is the study of all viruses

Don't consider each respiratory (gastrointestinal) virus in isolation

They interact when they meet

One virus, one disease – updated

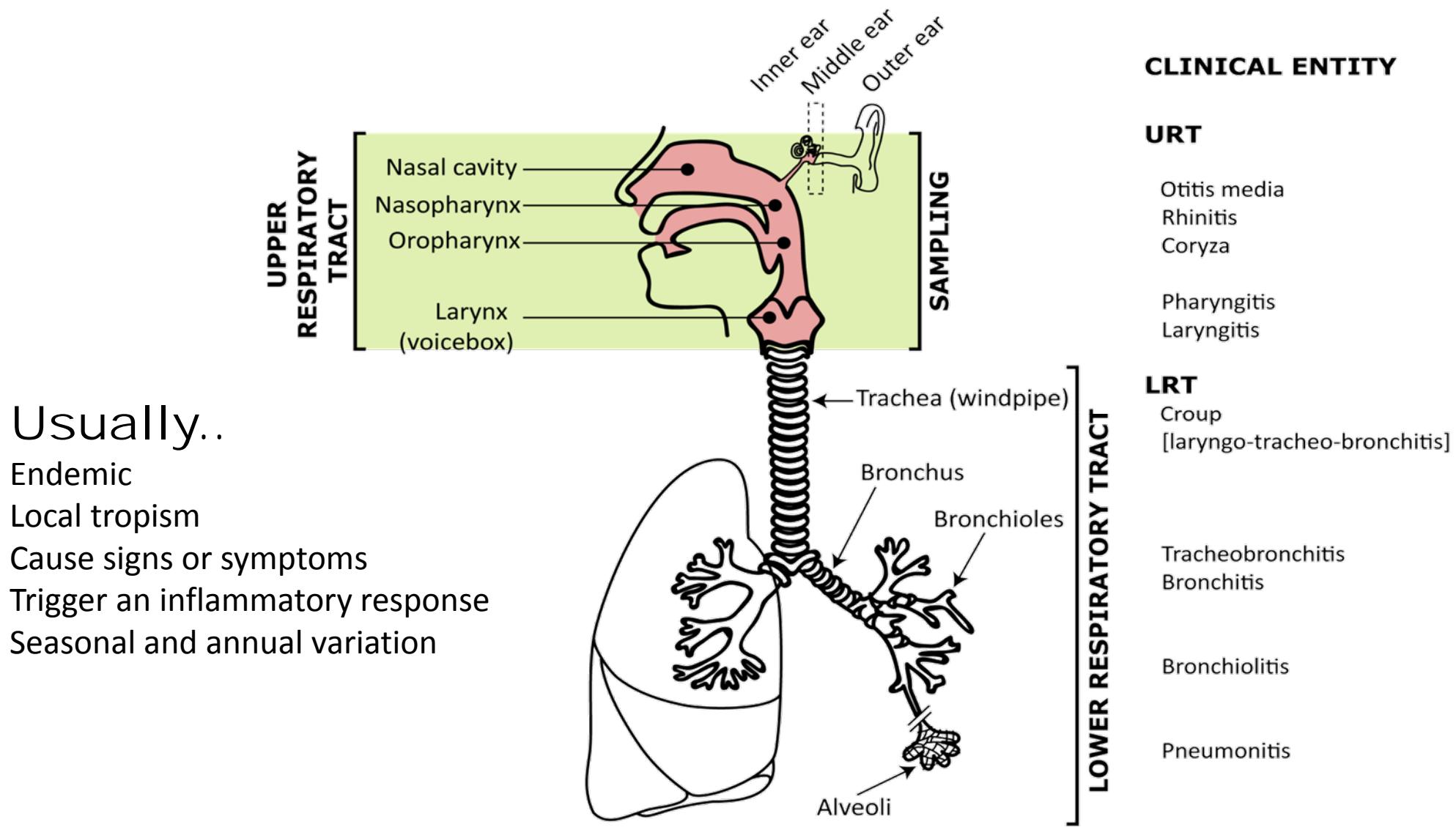
Huge challenges in virus:disease association (redefine the questions?)

HRV is not a single virus

160 different separate viruses

- Genetically different
- Antigenically different
- Seasonally different
- Clinically different?
- Immunobiologically different?

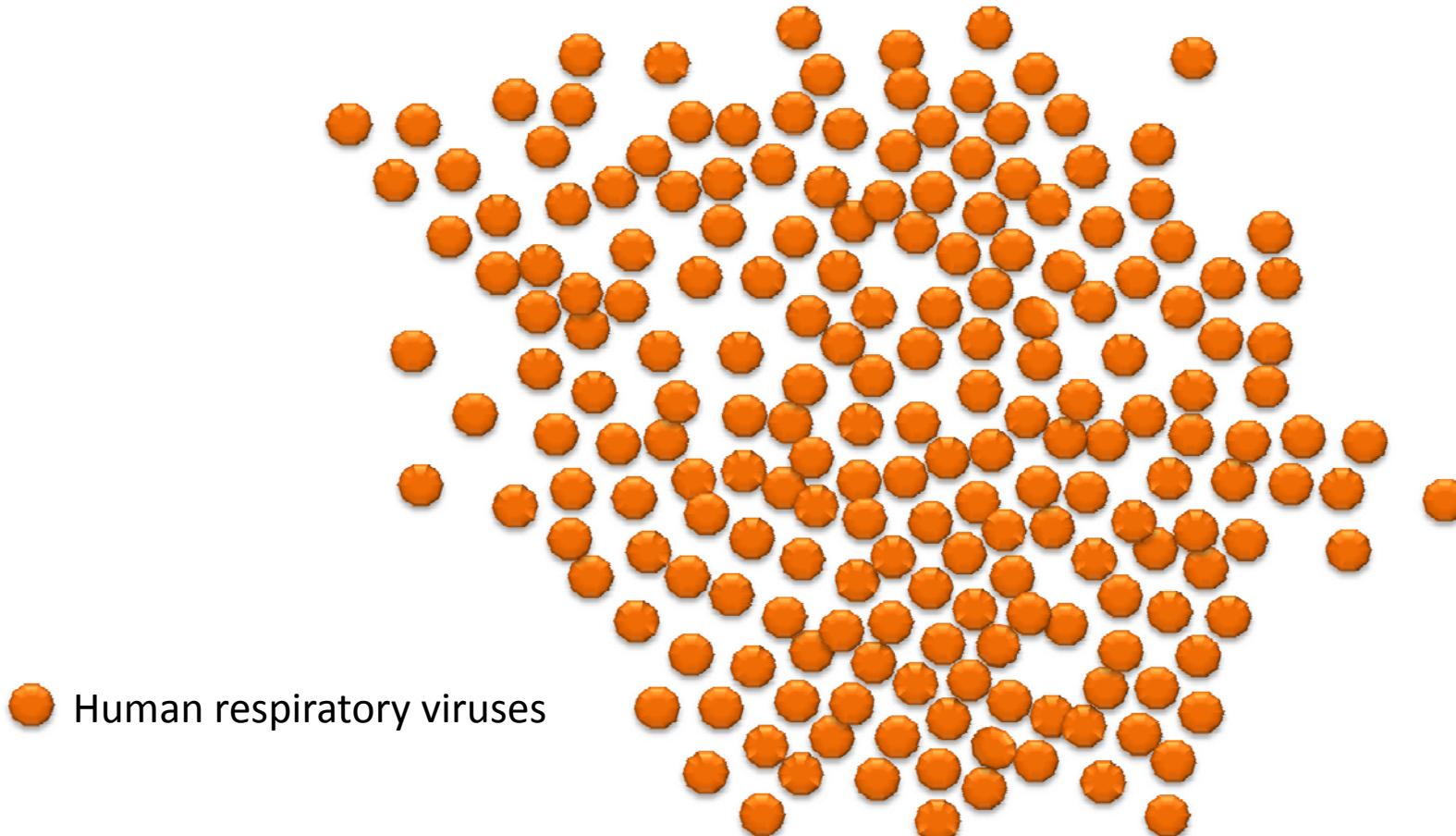
Respiratory virus



Usually..

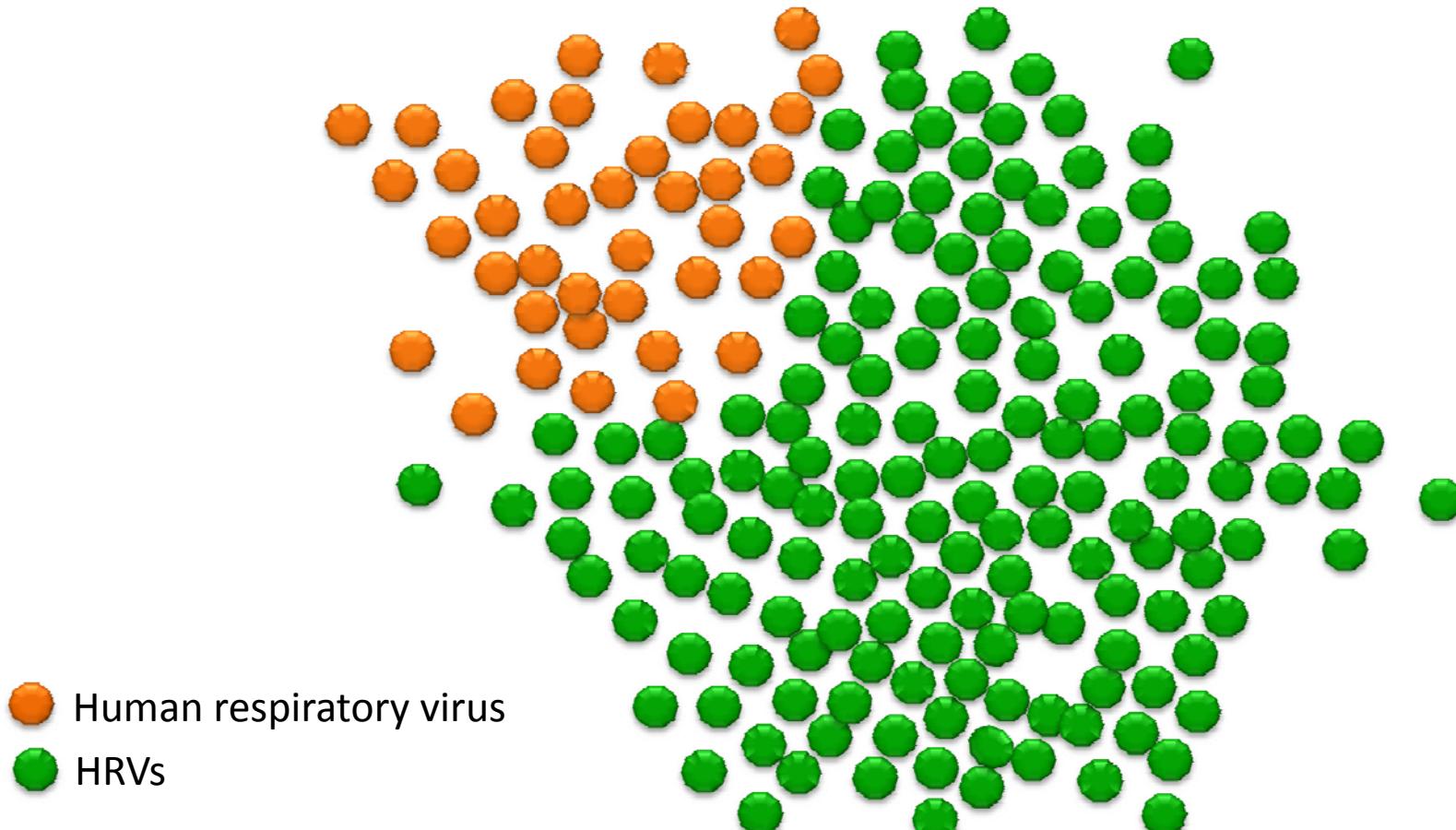
- Endemic
- Local tropism
- Cause signs or symptoms
- Trigger an inflammatory response
- Seasonal and annual variation

Distinct human respiratory viruses

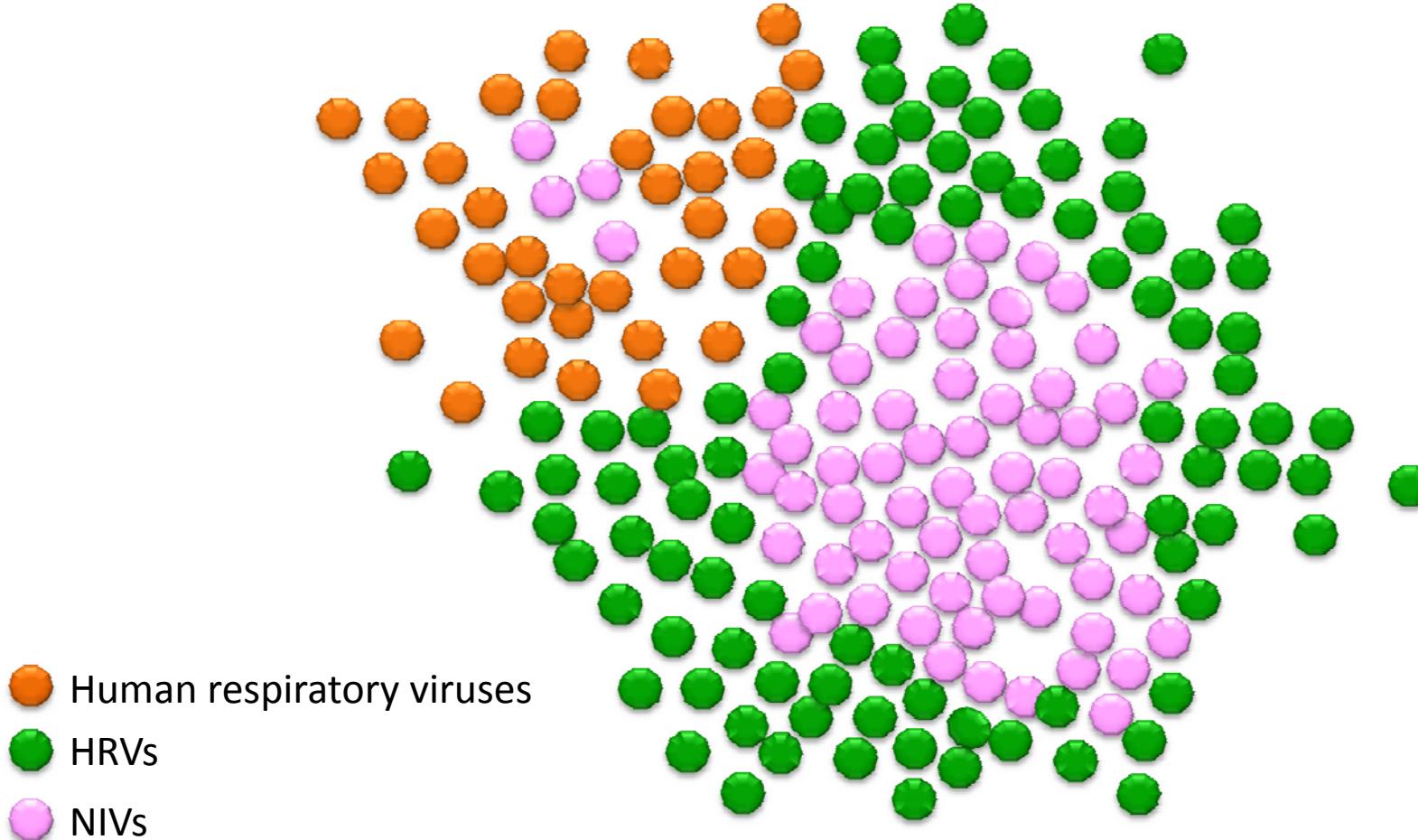


● Human respiratory viruses

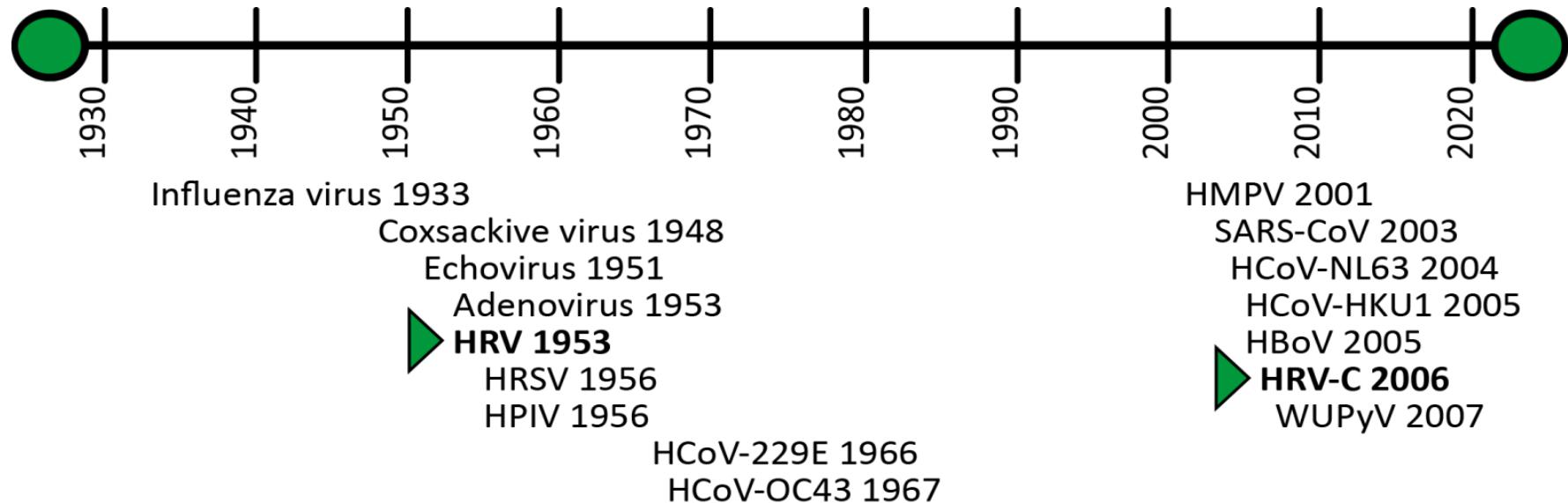
HRVs



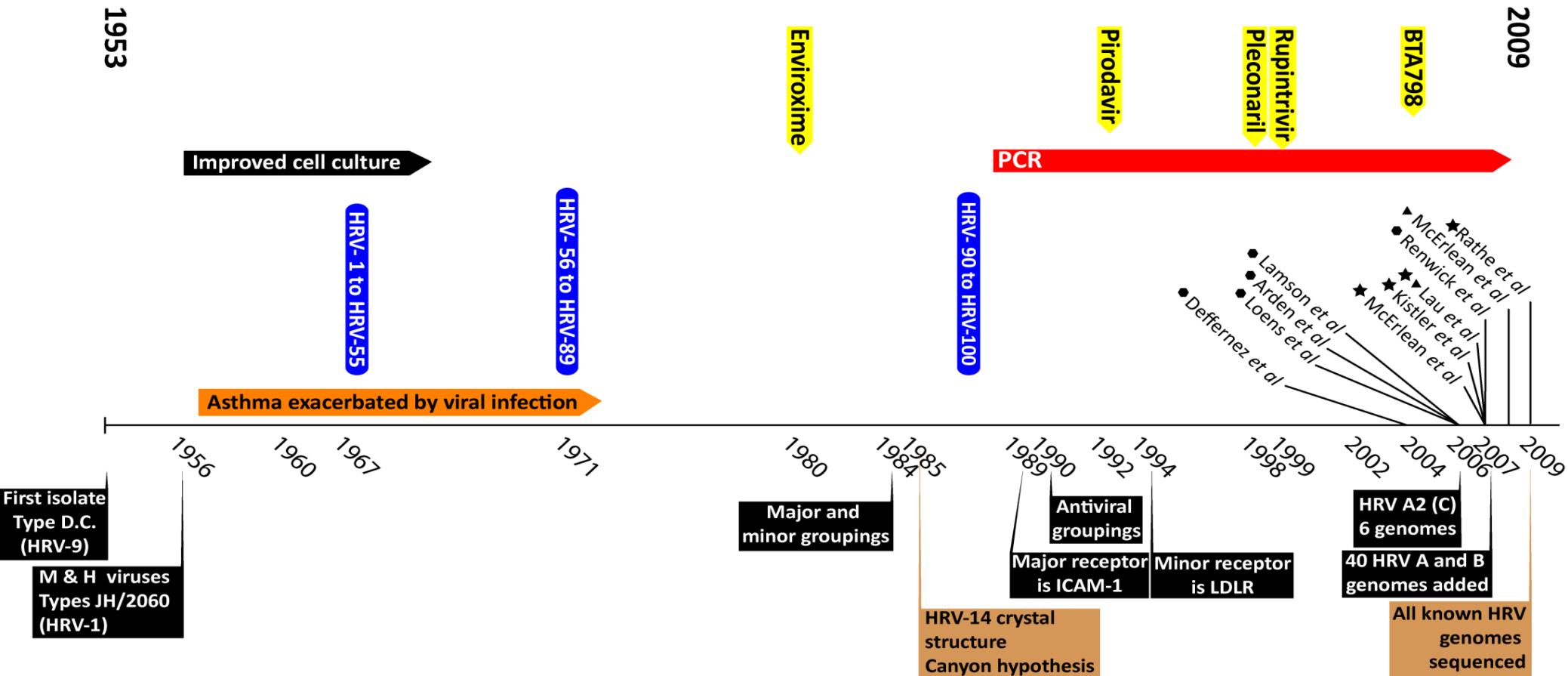
Newly identified viruses (NIVs)



HRV discovery



History of the HRVs



Human rhinovirus (HRV)

Human volunteer infection studies

Adults

Adult surveillance studies (culture-based)

~20% specimens HRV-positive

Polymerase chain reaction

1988

Quadrupled culture

Transmission

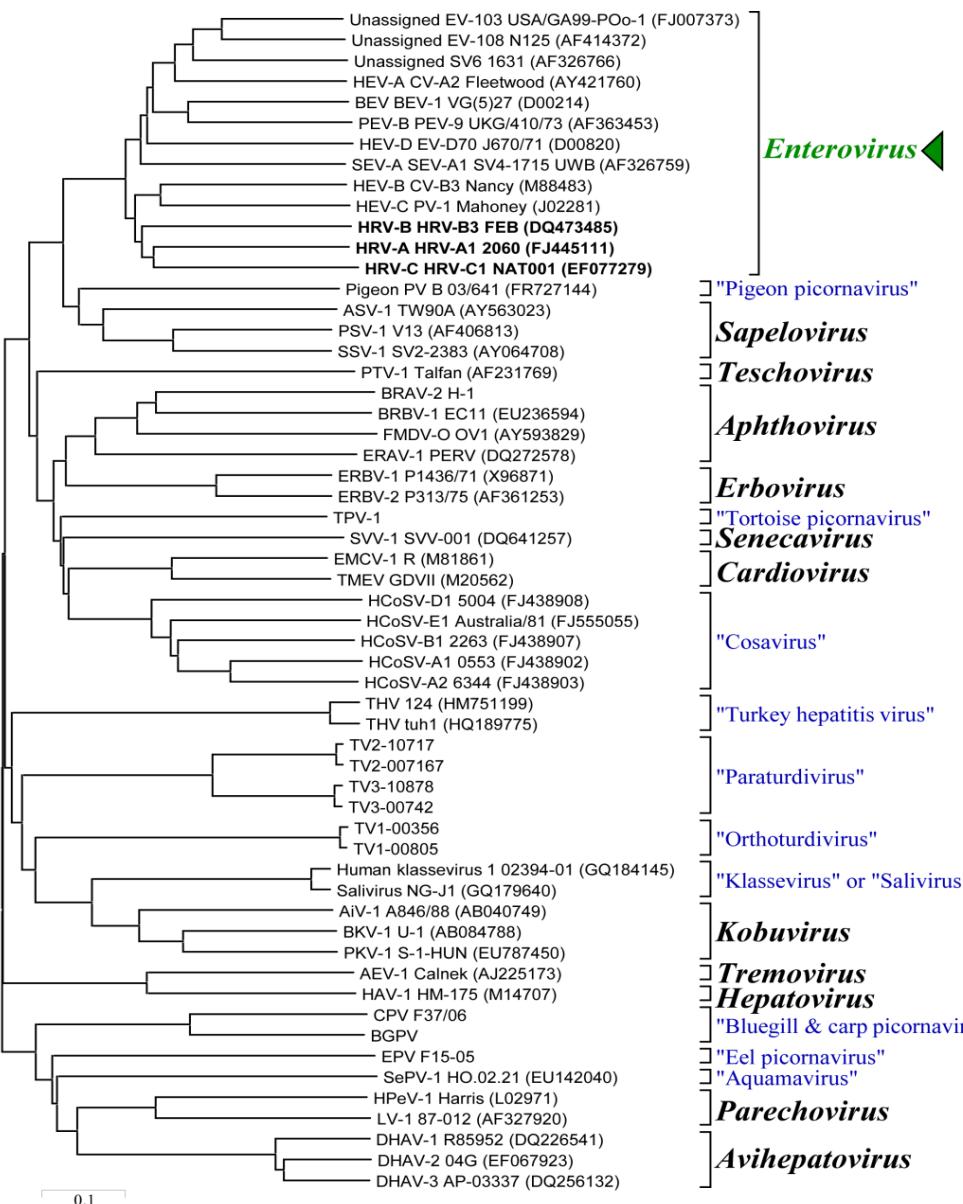
Fomites and self-inoculation

Aerosols

Persistent infections

No (confounded by overlapping, genotypically-distinct, infections)

Taxonomy of the HRVs



Family *Picornaviridae*

12 genera (>58% aa diff), ~80 species

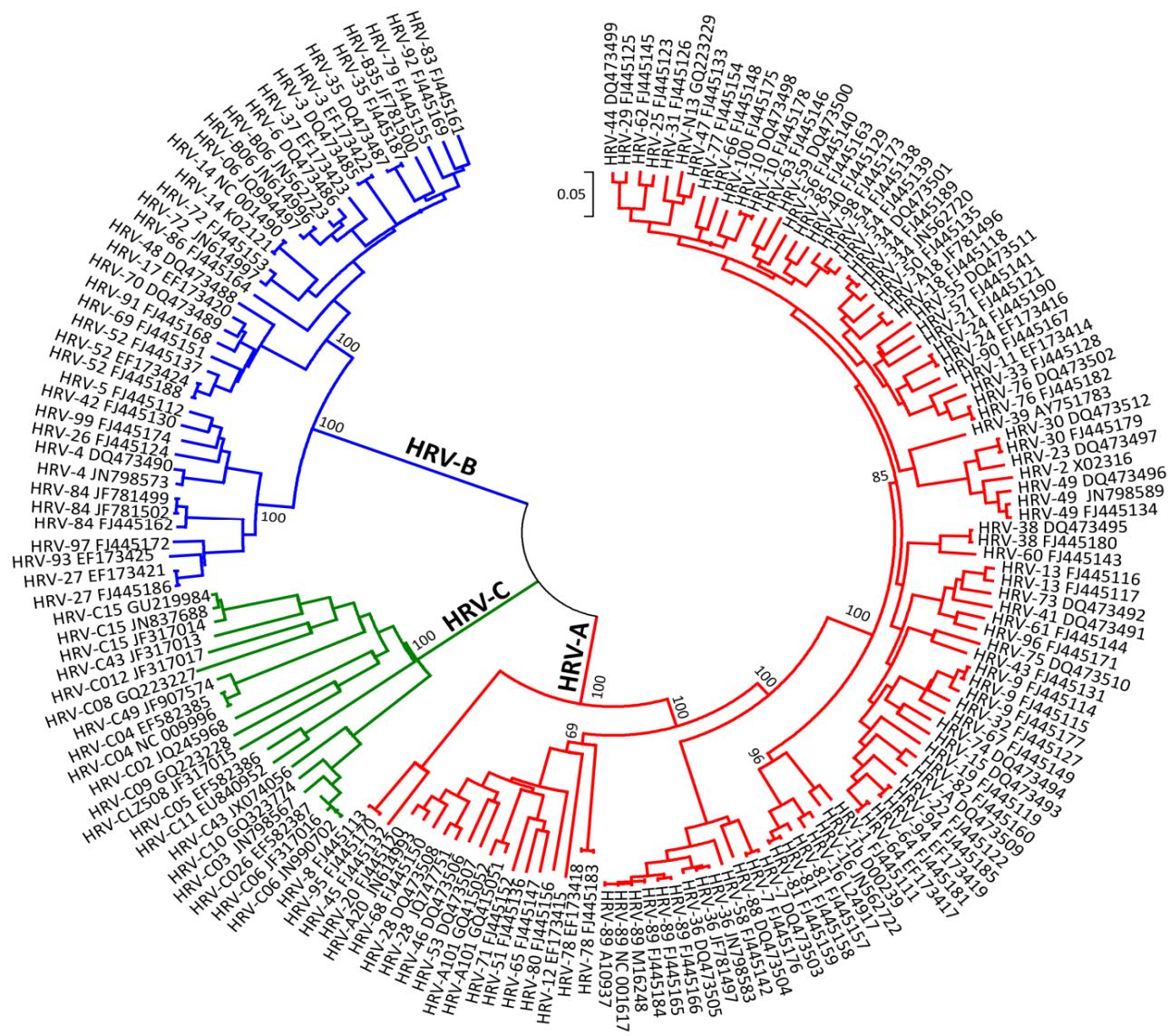
>360 types, >250 in human

Diverse clinical syndromes

Three species of HRV

- HRV-A: 75 serotypes
- HRV-B: 25 serotypes
- **HRV-C: 63 genotypes**

The July 2012 rhinovirome

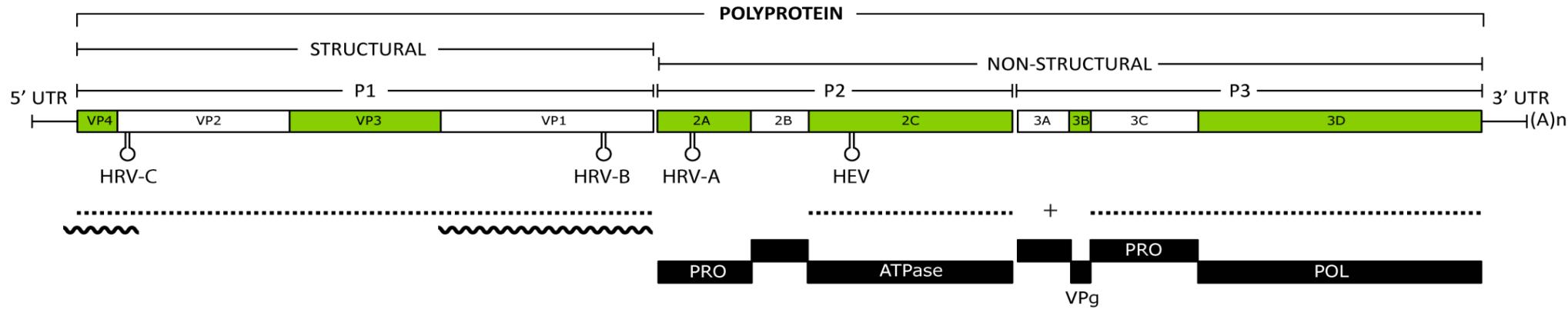


HRV genome

Encode a single polyprotein

Co- and post-translationally proteolytically cleaved

4 capsid peptides (VP1, 2, 3, 4)



Initial tropism of HRVs



HRVs replicate in:

- Nasal cells
- Sinus cells
- Bronchial epithelial cells
- Smooth muscle cells

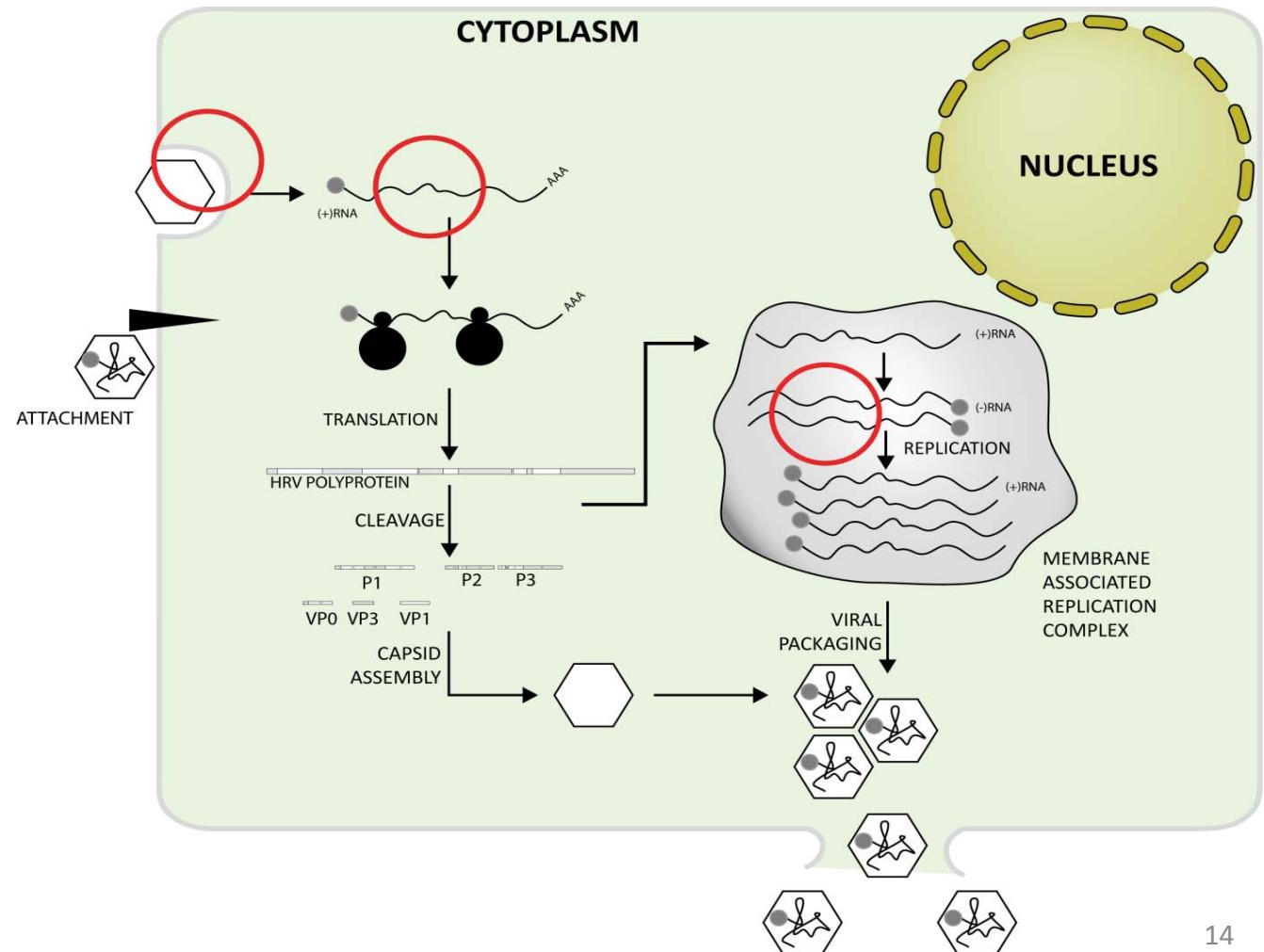
HRVs don't replicate in:

- Monocytes
- Dendritic cells

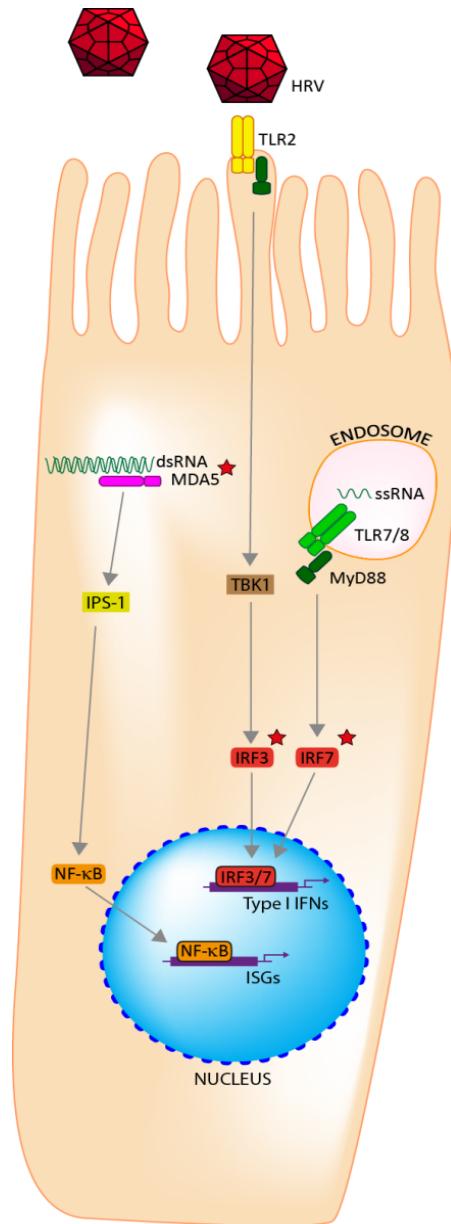
HRV replication and structure

~7kb ssRNA (+) genome
dsRNA intermediates

Small virion
~30nm
Non-enveloped



Early intracellular detection of HRVs



Innate

ssRNA, dsRNA, proteins recognized by:

- MDA5 (internal)
- TLR2/4 (external)
- TLR3/7/8 (endosomal)

Endocrine (same cell) & paracrine (neighbour cells) IFN circuit

Hundreds of IFN-stimulated genes

Modulated by most respiratory viruses

Not modulated by HRVs

- Inflammatory response
- Inflammatory disease

Clinical impact of HRVs

Acute respiratory illnesses (ARIs)

~80% of ARIs are viral

70% of those are HRV-related

Many/most cases presenting with “swine flu” were HRV-positive ILIs

Wheeze

Asthma (“attacks”), chronic obstructive pulmonary disease (COPD) exacerbations

>70% of exacerbations are HRV-positive (more than any other virus)

“Asthmatics” have a predisposition to more severe outcomes from infection

Other complications

Otitis media

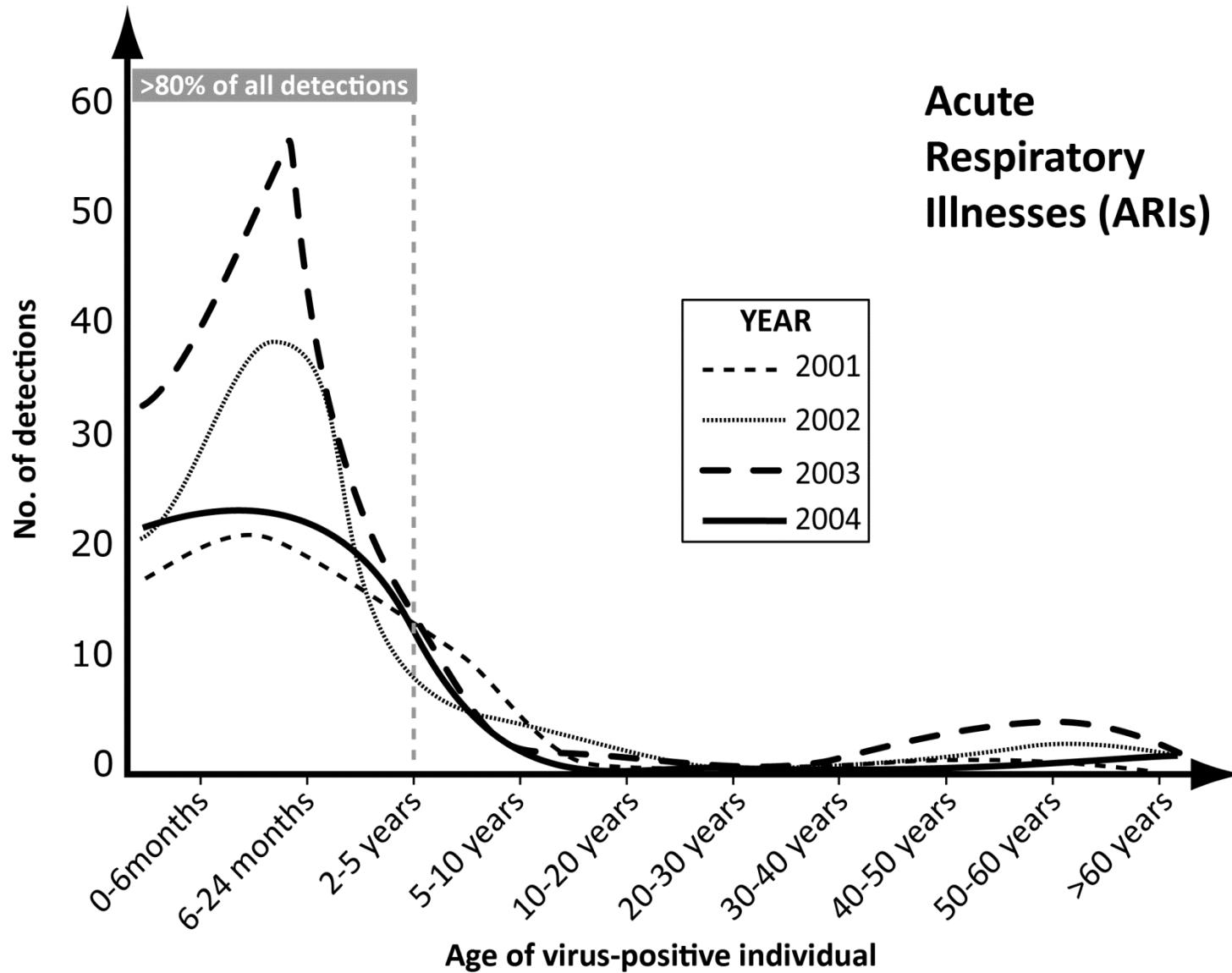
Bronchiolitis

Viral pneumonia/Secondary bacterial pneumonia

Acute gastroenteritis

Children bear the brunt of ARIs

Hospital-based, retrospective



Burden of disease

Respiratory infection/Respiratory diseases

Among top 5 causes of deaths, worldwide, 2004¹

ARIs lead in causes of death among children <5yrs²

Increase risk with indoor smoke, poor breastfeeding, underweight, urban pollution²

Asthma (exacerbations)

Top cause of disability-adjusted life years (DALYs) in Australian 0-14year olds²

HRVs frequently trigger exacerbations (80% of cases)

HRVs also cause wheeze in those without asthma

Multiple infections each year

13 symptomatic respiratory illnesses per year; 6 HRVs per year

Underestimate!

Time off school, work, GP visits, antibiotics, OTC drugs, hospitalization, asthma

Detecting HRVs?

How have we routinely detected the HRVs?

People, culture, RT-conPCR, RT-rtPCR, +/- RNA preparation, +/- bead-binding
HRV-Cs are not culturable in cell lines (HRV studies pre-1988 miss 60 HRVs)

Do all HRV PCRs detect all HRV types?

No

Earlier PCR-based assays target HRV-As and HRV-Bs

Several RT-rtPCRs work well

Cross-reaction between HRV and EV

Not always included in diagnostic menus

PCR is costly – balance against the number of new positives

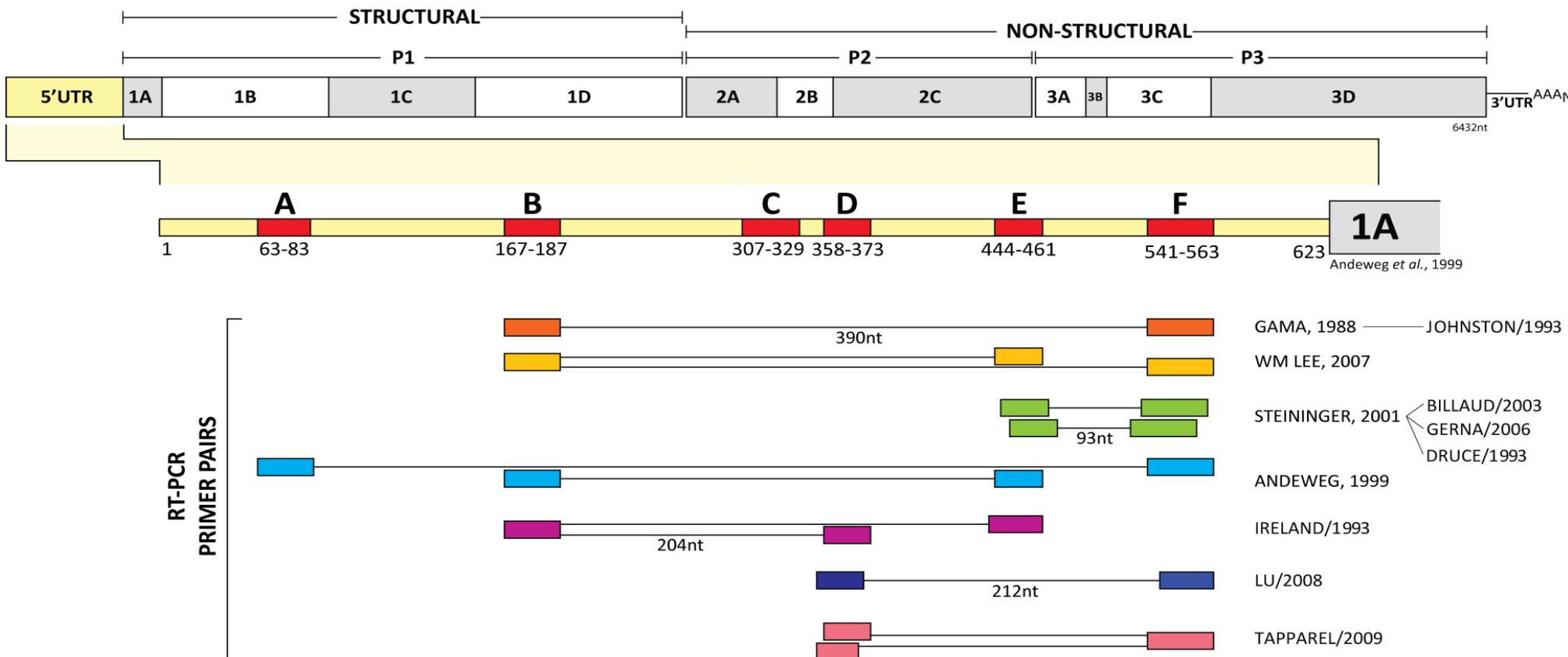
Commercial extended panels

Clinical benefit of knowing its an HRV? We already test for HRSV, HMPV, IFVs, HAdVs, HPIVs...

Not testing? Start the conversation

PCRs for HRV detection

5'UTR-based
Conserved sequence “islands”

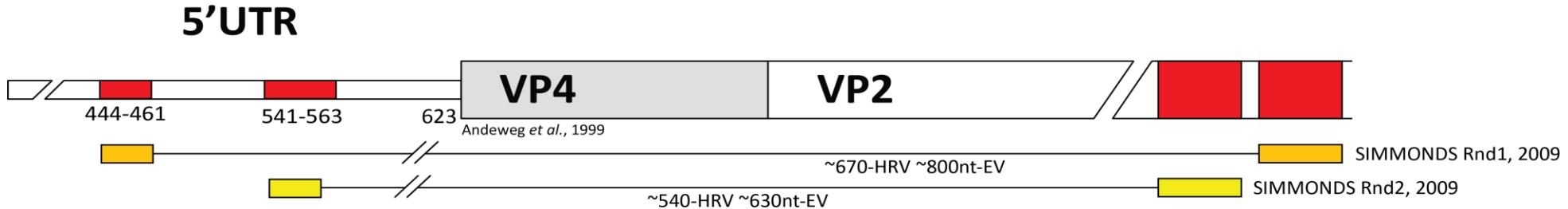


PCRs for genotyping the HRVs

VP4/VP2 region most widely used

Agrees with VP1 and complete genome phylogeny (gold standards)

UTR – only with experience



Improved characterization: impact

Short term

Limit invasive searches

Reduce family stress

Better target antibiotic use (for bacteria, pneumonia)

Better cohorting of inpatients

Longer term

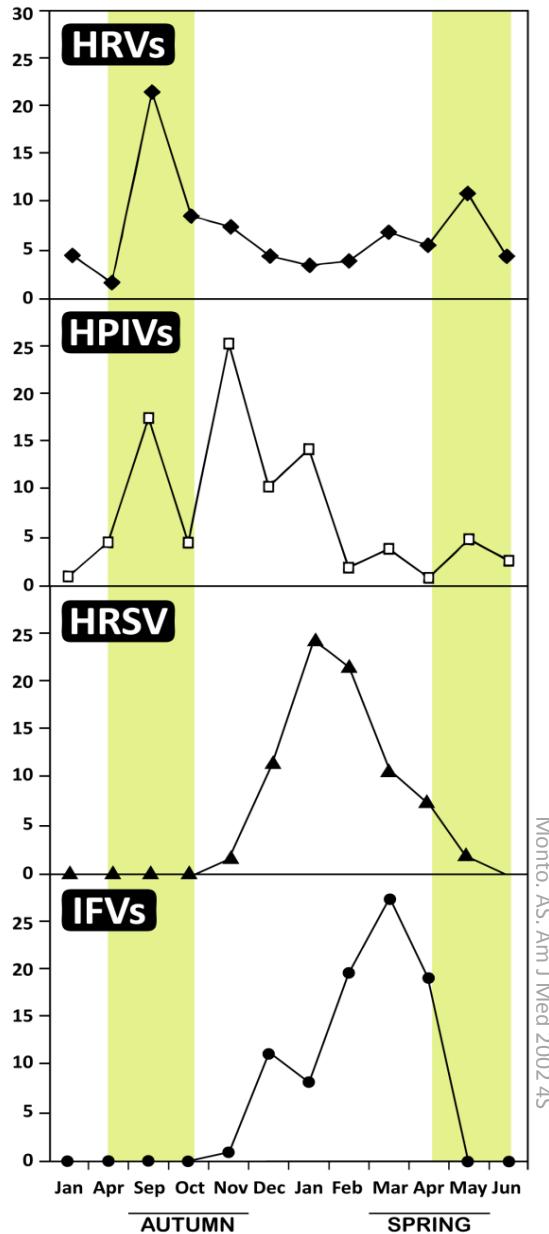
Understanding and treating human disease and the human virome

Improved epidemiology - pandemic preparedness (better use of antivirals, vaccines?)

New knowledge

Prioritize antiviral and vaccine developments

HRV seasonality: pre-PCR



Cell culture data (e.g. Michigan, US)

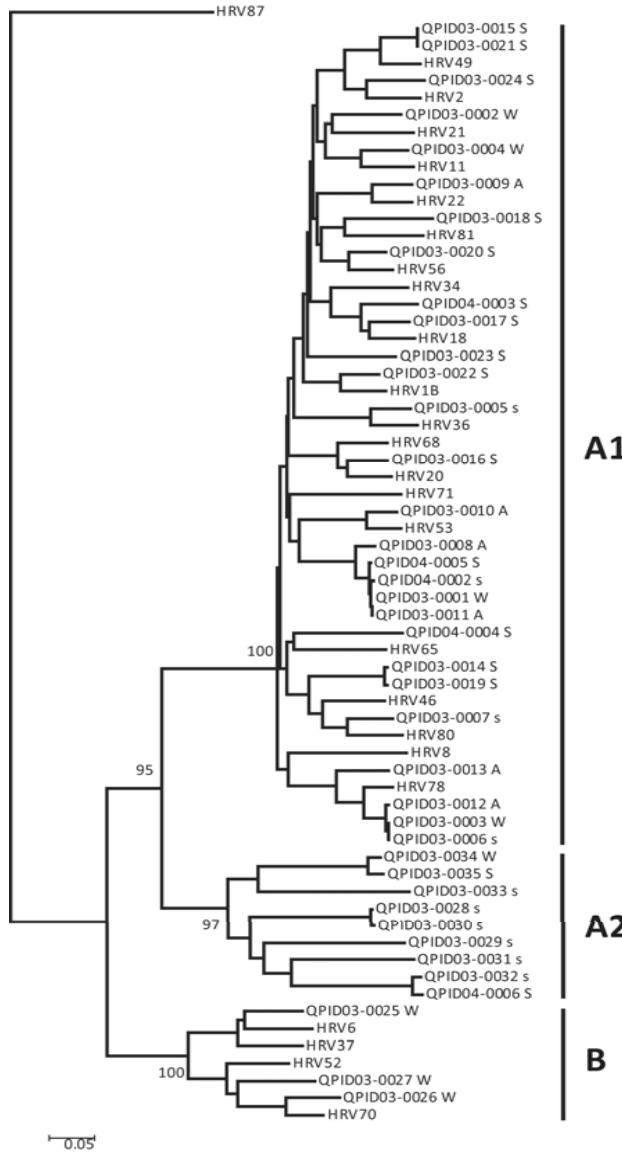
Bimodal peak

Excludes HRV-Cs

Bracket cytopathic virus peaks - which is dominant?

- Don't know

Discovering a new HRV species, HRV-C



A1

Dsitinct cluster of sequences
Hospital-based, retrospective, PCR
Specimens from 2003 (2006)
HRV-A2

A2

B

A novel species, HRV-C

Complete novel coding sequence

HRV-C3 (f.QPM), first distinct HRV in 20 yrs (2007)

Human rhinovirus C

Global distribution

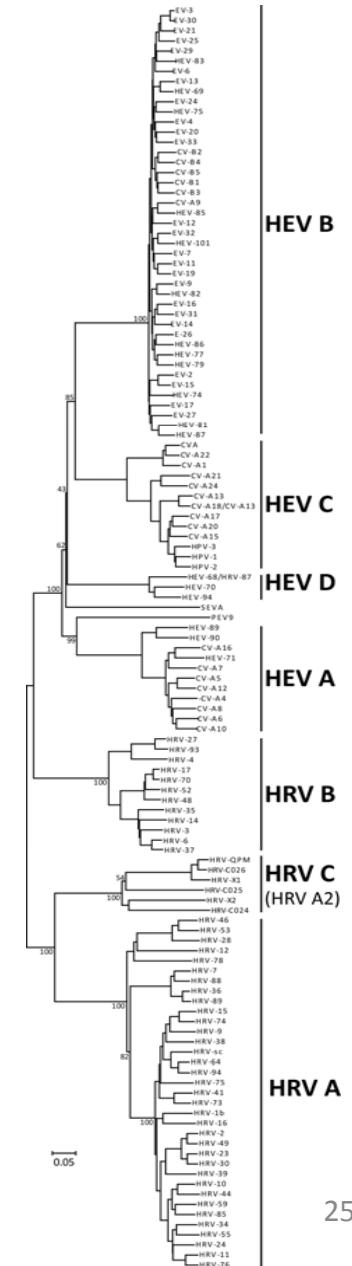
Global failure to culture

At least in cell lines

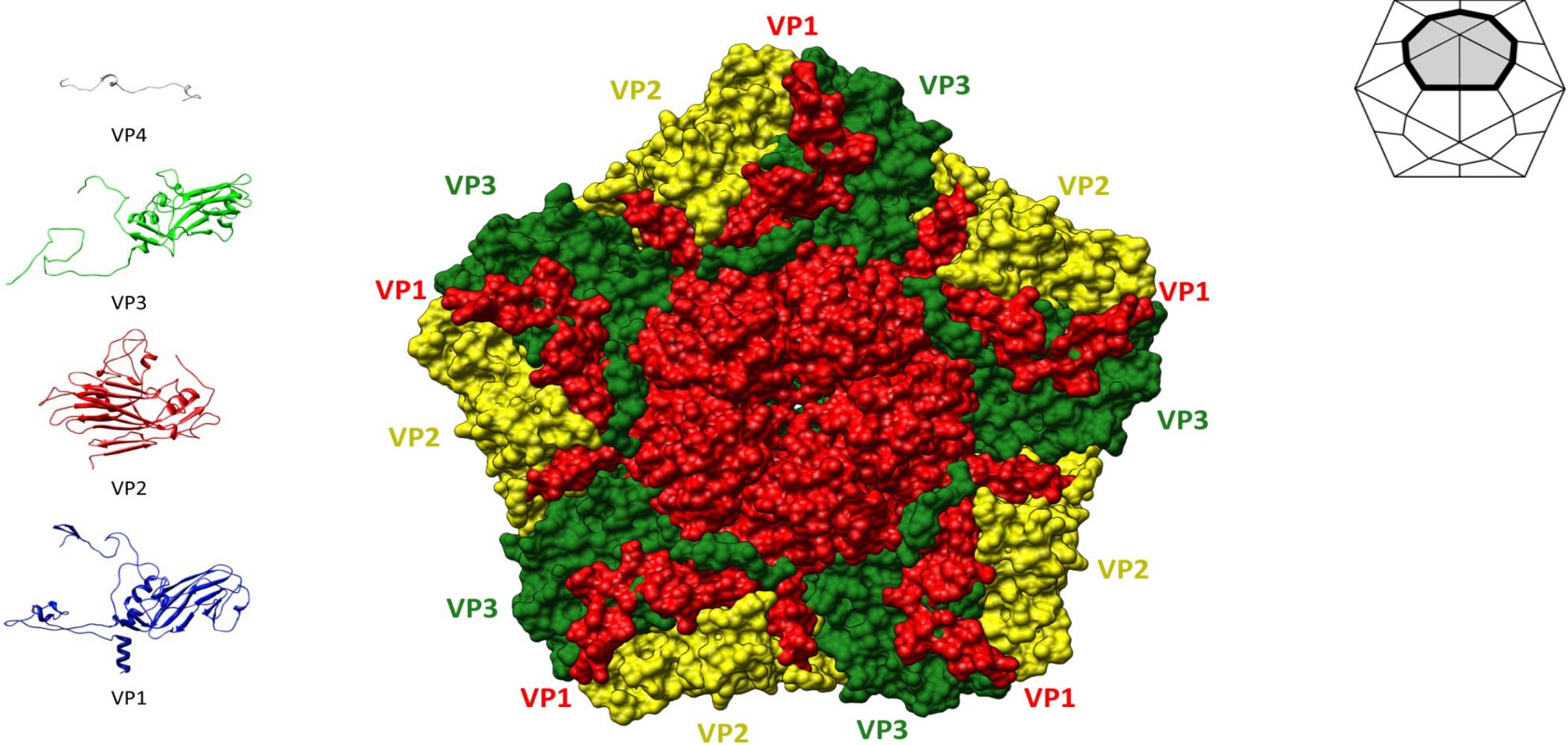
Recently isolated using sinus organ culture

How do you study an unculturable virus?

- Reverse genetics (infectious clones)
- *In silico*



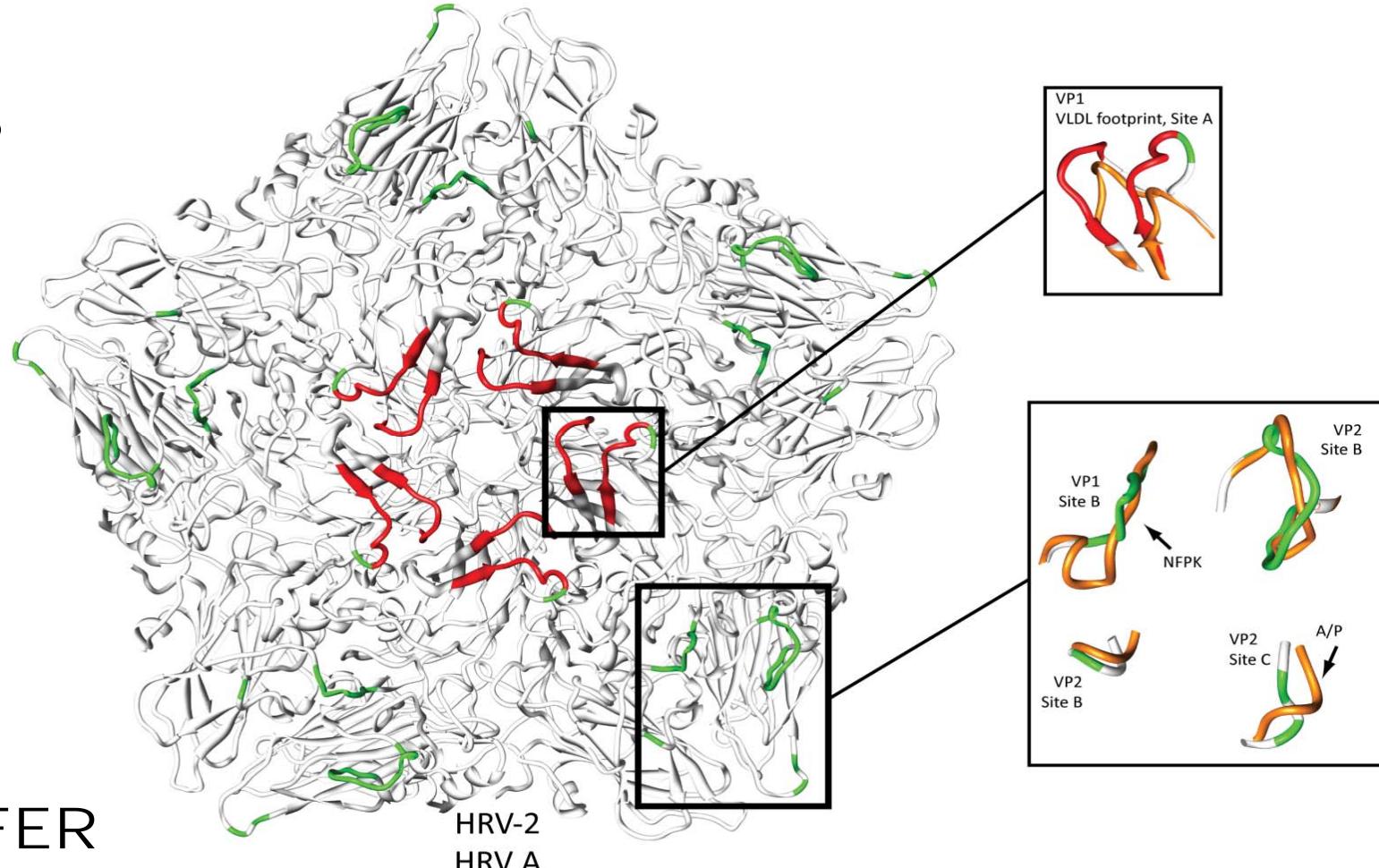
Predicting an HRV-C structure



Following the footprints

RECEPTORS

ICAM-1 (major group)
VLDL-R (minor group)



HRV-Cs DIFFER

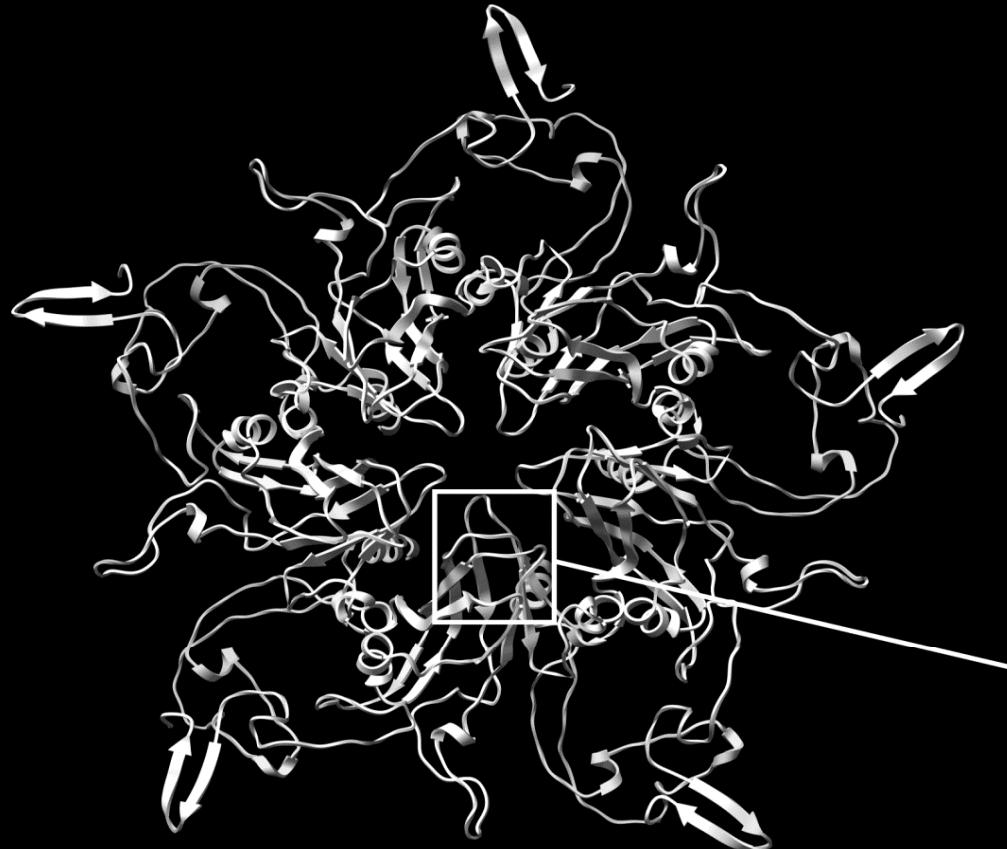
Comparative structure

Green-HRV-2 antigenic sites

Red-known minor group receptor binding footprint

It doesn't take much to be different

A



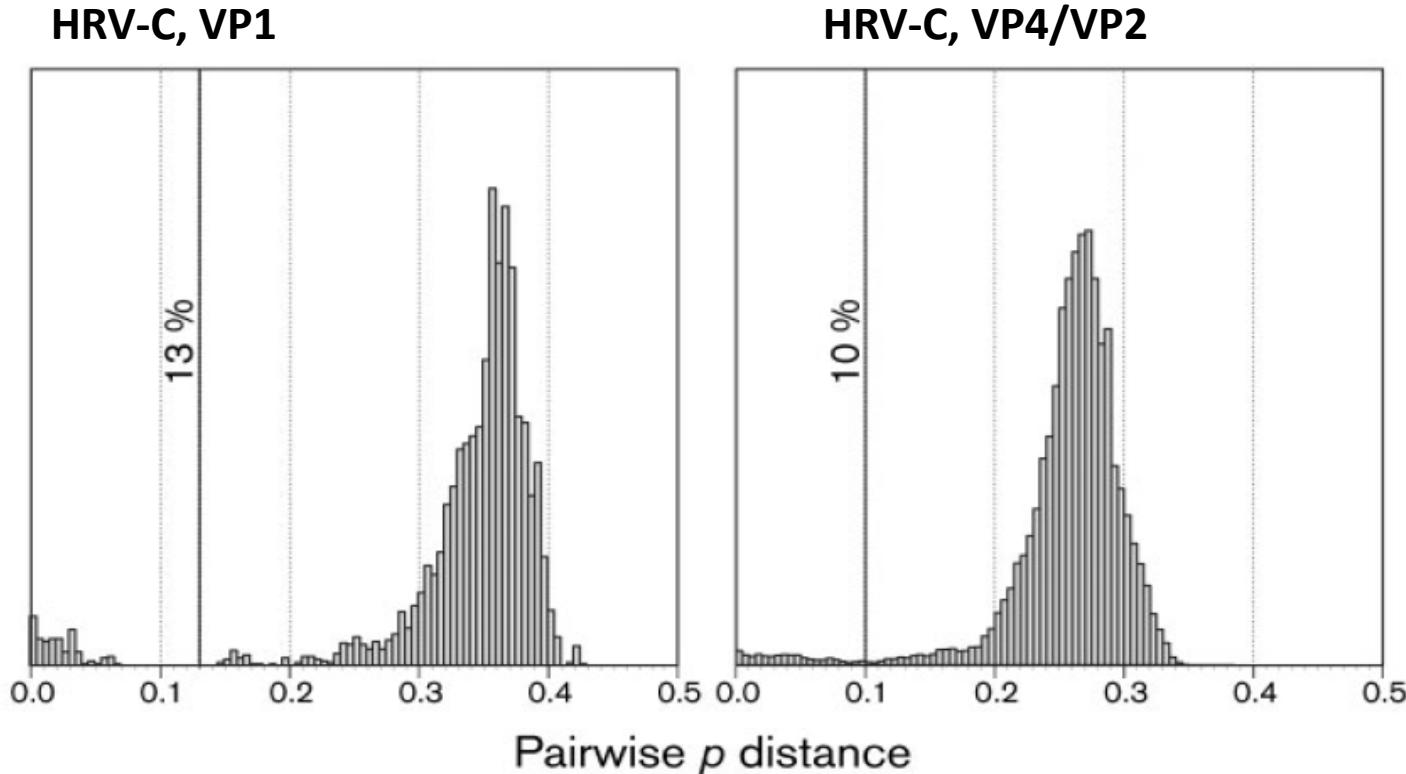
HRV PENTAMER
Bird's eye view

B



HRV PROTOMER
1/60th of capsid

HRV-C: defining a species



Same, same, difference

>13% nt divergence in VP1 → a distinct, novel HRV-C type

>10% nt divergence in VP4/VP2

HRV nomenclature

Rhinovirus

A	B	C
<u>1</u> ^{M,B}	34^B	64^B
<u>2</u> ^{M,B}	36^B	65^B
<u>7</u> ^{H,B}	38^B	66^B
<u>8</u> ^{H,A}	39^B	67^B
<u>9</u> ^{H,B}	40^B	68^B
10^{H,B}	41^B	71^B
11^{H,B}	43^B	73^B
12^{H,B}	44^B	74^B
13^{H,B}	45^B	75^B
15^{H,B}	46^B	76^B
16^{H,B}	47^B	77^B
18^{H,B}	49^B	78^B
19^{H,B}	50^B	80^B
20^{H,B}	51^B	81^B
21^{H,B}	53^B	82^B
22^{H,B}	54^B	85^B
<u>23</u> ^{H,B}	55^B	88^B
24^{H,B}	56^B	89^B
<u>25</u> ^{H,B}	57^B	90^B
28^{H,B}	58^B	94^B
<u>29</u> ^{M,B}	59^B	95^A
<u>30</u> ^{M,B}	60^B	96^B
<u>31</u> ^{M,B}	61^B	98^B
32^B	62^B	100^B
33^B	63^B	N13
		99^A
		C3 (f. QPM)
		C10 (f. QCE)
		C1 (f. NAT001)
		C2 (f. NAT045)
		C4 (f. C024)
		C5 (f. C025)
		C6 (f. C026)
		C7 (f. NY074)
		35 ^A
		37 ^A
		42 ^A
		48 ^A
		52 ^A
		69 ^A
		70 ^A
		72 ^A
		79 ^A
		83 ^A
		84 ^A
		86 ^A
		91 ^A
		92 ^A
		93 ^A
		97 ^A
		99 ^A
		C26
		C27
		C28
		C29
		C30
		C31
		C32
		C33
		C34
		C35
		C36 (f. NAT069)
		C12
		C37 (f. NAT059)
		C13
		C38 (f. tu34)
		C14
		C39 (f. g2-11)
		C15
		C40 (f. g2-25)
		C16
		C41 (f. g2-23)
		C17
		C42 (f. g2-28)
		C18
		C43 (f. 06-230)
		C19
		C44 (f. PNC40168)
		C20
		C45 (f. PNC40449)
		C21
		C46 (f. KR2315)
		C22
		C47 (f. K1091_301104)
		C23
		C48 (f. PNG7293-3193)
		C24
		C49 (f. IN-36)
		C25
		C50 (f. SG1, SO5986)

How many stable HRVs still circulate?

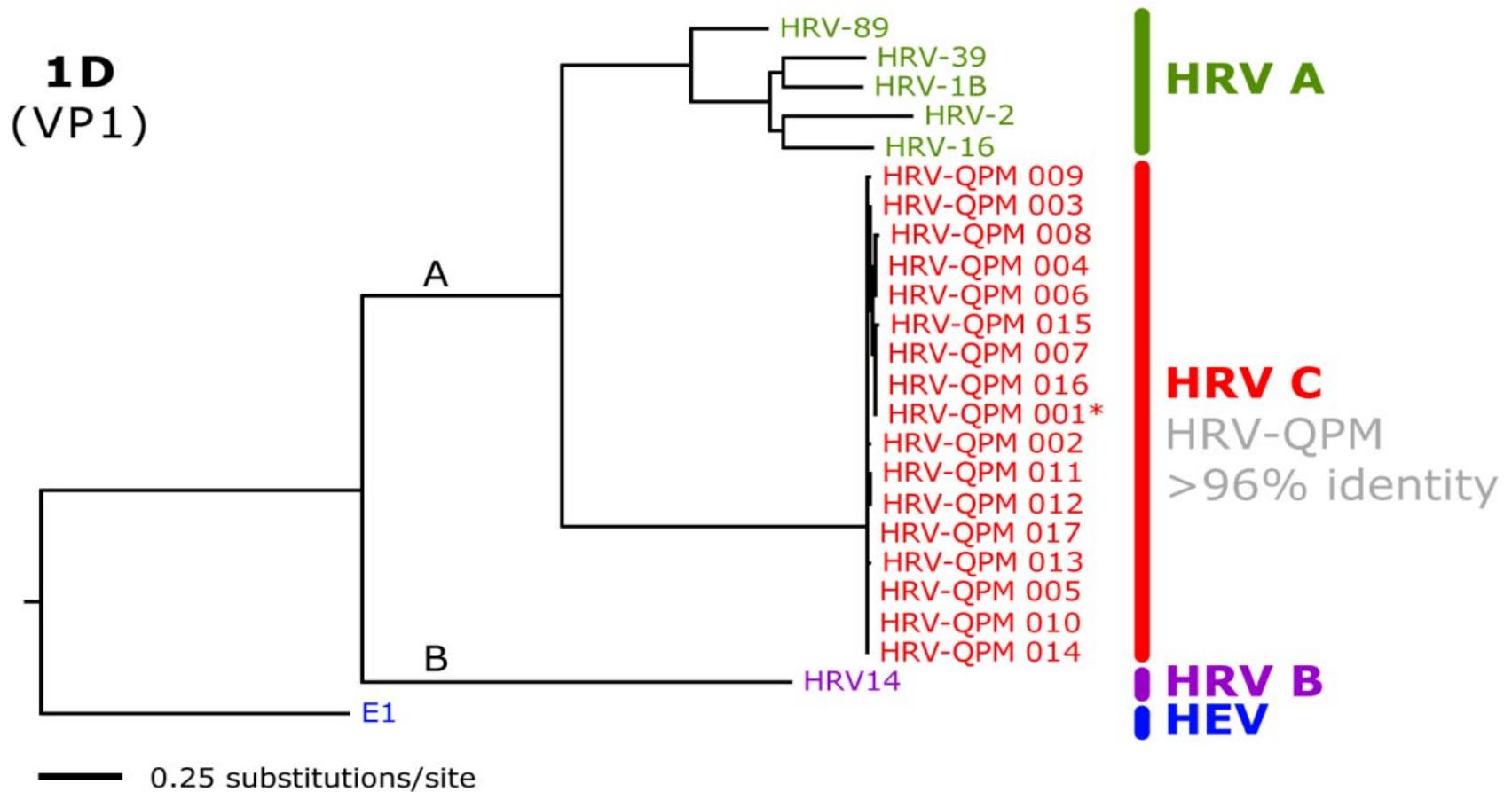
~70 types co-circulate

During periods of peak HRV activity across a year
Hospital or community

HRVs are under positive selective pressure

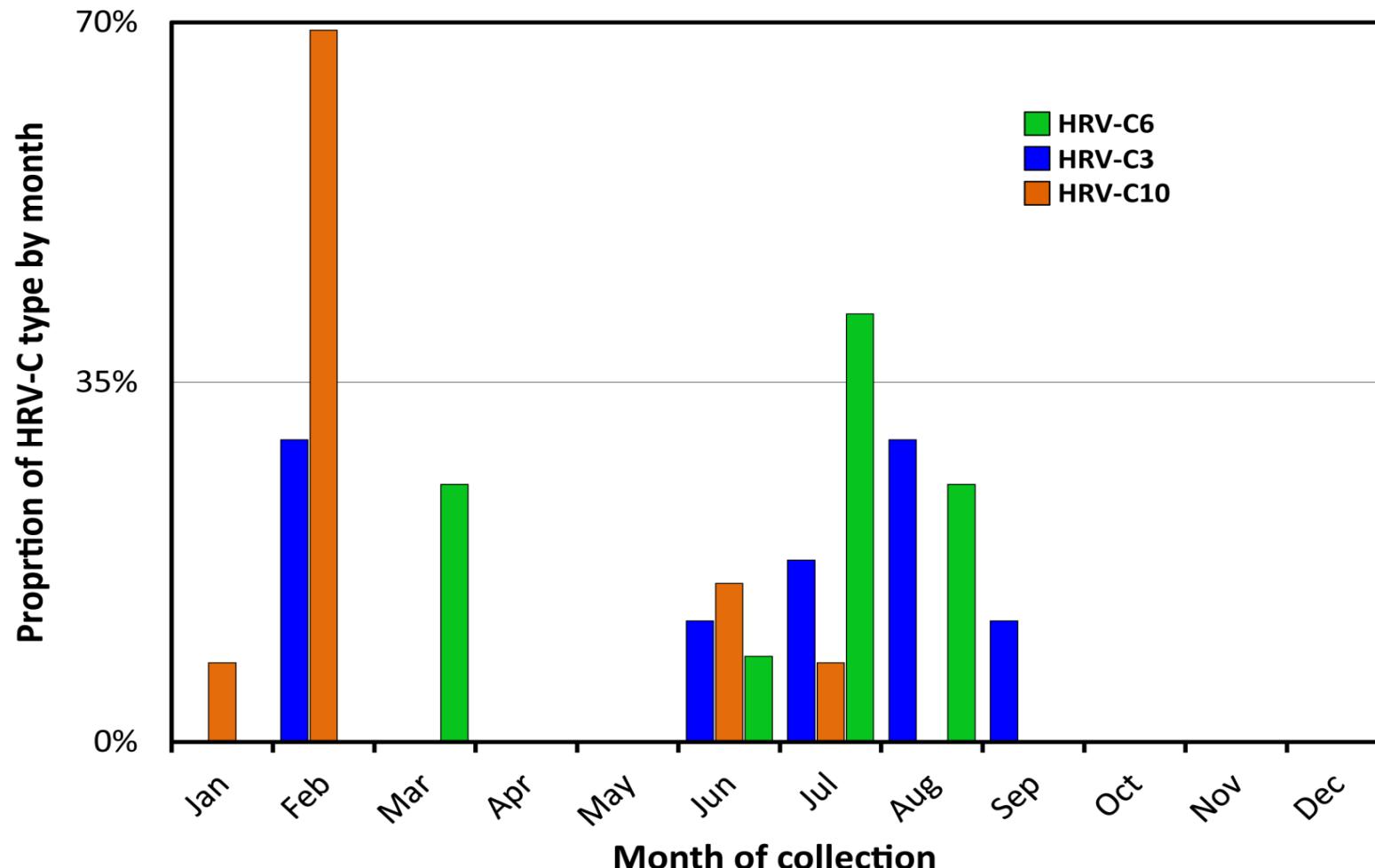
Small changes to antigenic sites under pressure (antiviral?)
A type, is a type, is a type – stable genomes
New types emerging (impact of a new type)?

How stable is an HRV type?



Confirming the bimodal distribution

Single HRV types; highly specific RT-rtPCR



x80

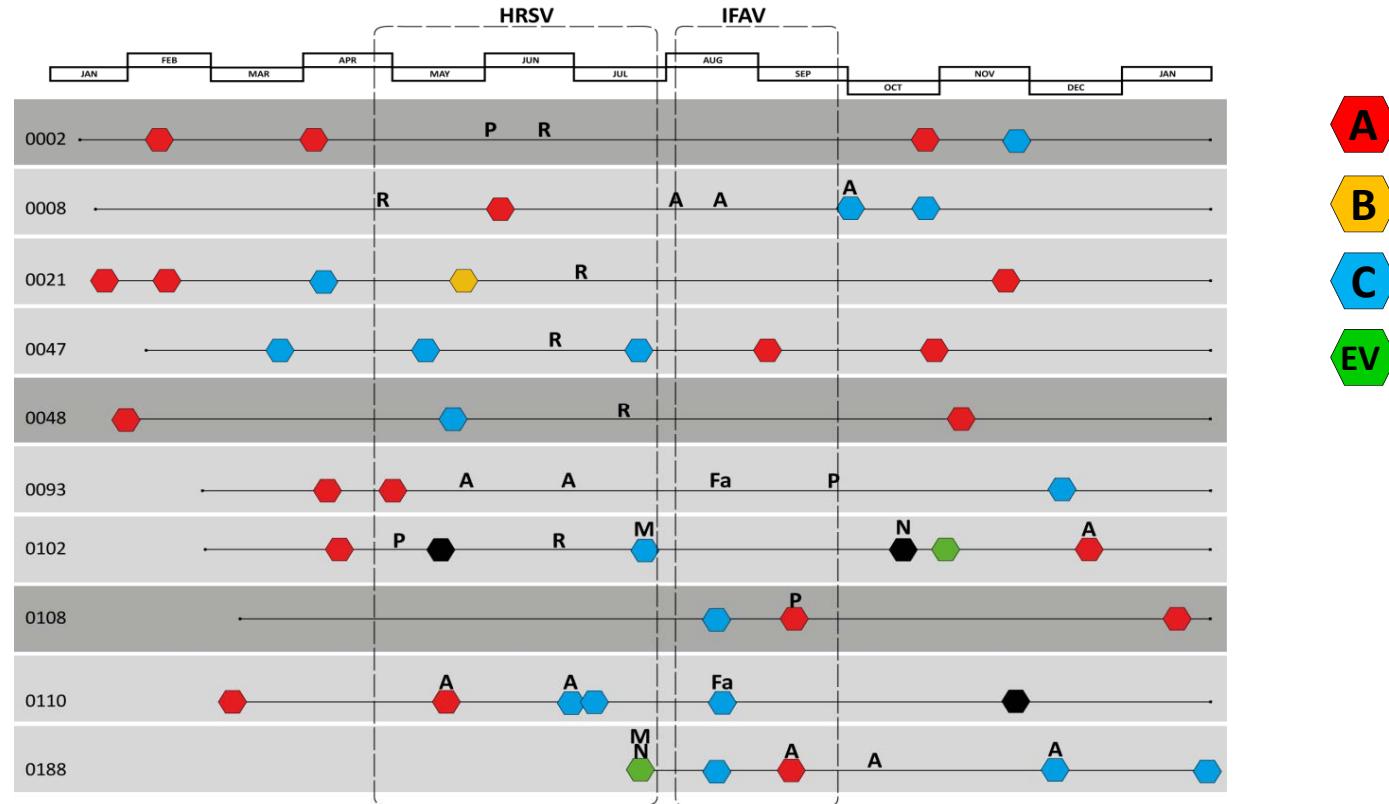
160 HRV types

HRV-C3 (f.QPM) clinical impact

Diagnosis and HRV-C3 variant	Age	Other viruses	Severity Score
EXPIRATORY WHEEZING (n=9, 52.9%*)			
HRV-C3 001 ^a	10 months	— ^b	2
HRV-C3 012	5 months	—	4
HRV-C3 010	10 months	—	2
HRV-C3 004	27 days	—	0
HRV-C3 006	7 months	—	2
HRV-C3 003	2 years 8 months	—	1
HRV-C3 005	67 years 11 months	—	NC
HRV-C3 002	3 years 7 months	—	NC
HRV-C3 007	4 years 7 months	—	1
PERSISTENT / HACKING / WHOOPING COUGH (n=5; 29.4%)			
HRV-C3 014	9 months	—	0
HRV-C3 013	1 year 4 months	HBoV; WUPyV	1
HRV-C3 016	11 months	HMPV	1
HRV-C3 017	11 months	NL 63	3
HRV-C3 015	1 month	NL 63	2
FEBRILE CONVULSION (n=1; 5.9%)			
HRV-C3 0011	2 years 6 months	—	1
UNDERLYING CONDITION (n=2; 11.8%)			
HRV-C3 009	29 years 9 months (CF deterioration)	229E	NC
HRV-C3 008	3 years 7 months (CHD; URTI)	HBoV, NL 63	NC

* Percentage of positives. ^a HRV-QPM strain detected. ^b Negative after all viral investigations (n=17). NC No chart available

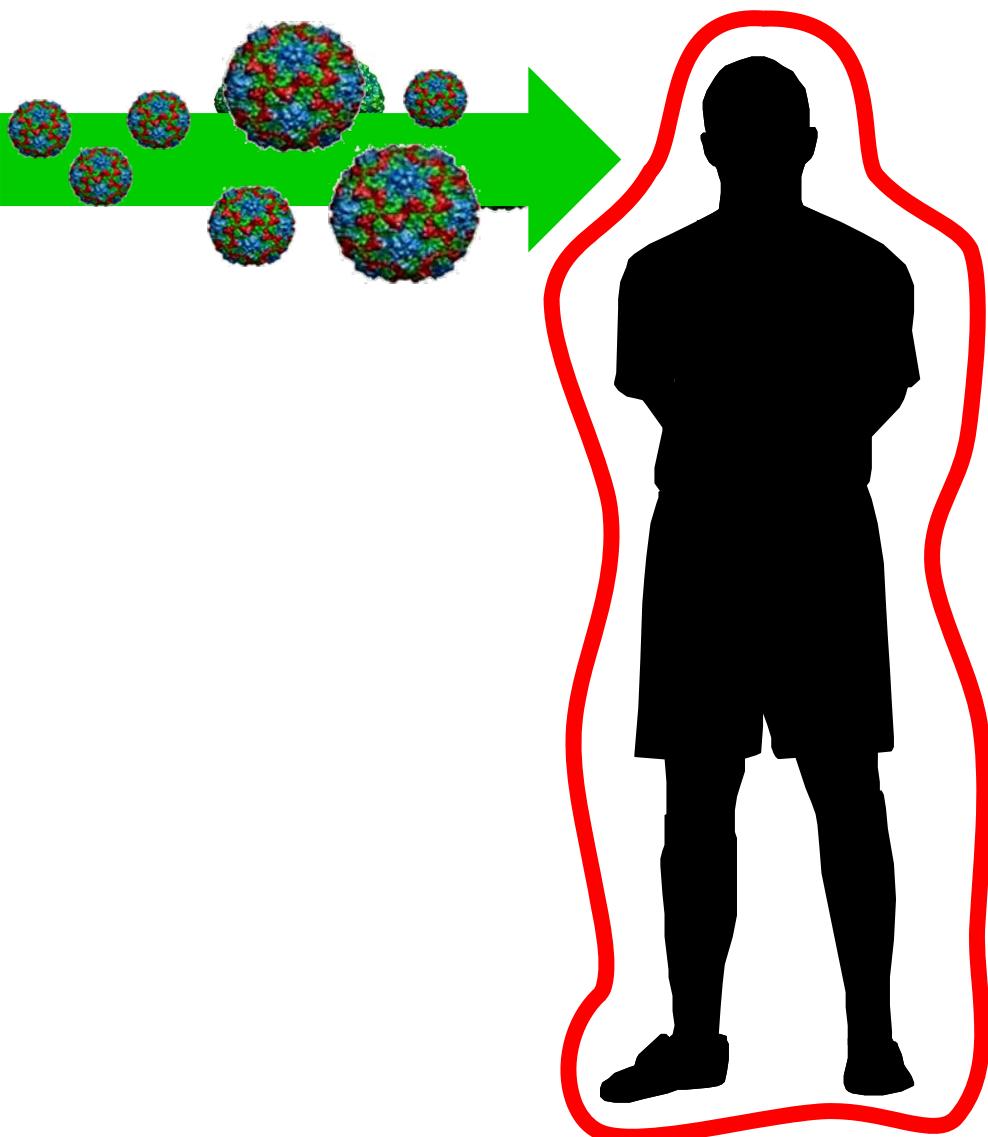
Number of HRVs per year



Community Cohort

Victoria (Stephen Lambert and Terry Nolan)
2003, children followed for 12 months
Up to 6 HRV POS SIRIs/yr

Protective effect of infection



Far better data

PCR sensitivity

Inclusion of HRVs

Patterns emerged

Co-detections

- HRVs significantly under-represented

Seasonality

- Viral seasons are distinct
- Not so with emerging virus

Protective virus

RNA viruses triggering an antiviral state

Especially the HRVs

Less so for viruses that block host response

Hypothesis

HRVs are a protective environmental factor
(among non-asthmatics)

Still to learn

What don't we know about the HRV-Cs?

Are HRV-Cs the asthmagenic HRVs?

Receptor(s)

Antiviral efficacy (Vapendavir) how soon will resistance develop?

Immunobiology

Antigenic sites

Gut tropism (AGE detections, high loads)

HRV-C virome

Conclusions

HRVs predominate in ARIs

70 types circulate at a single site, mix varies

Types are distinct and conserved

HRV species exchange dominance

Cross sectional, disease specific studies miss this

RNA viruses under-represented in CoDes.

Infection may render the host less susceptible to super-infection

Could repeated HRV exposures from many immunogenically discrete viruses protect the host from more “severe” viruses?

Influenza – the chicken or the egg?

A rise in IFAV cases prelude a precipitous decline in HRV cases

IFAV creates the bimodal distribution attributed to HRV epidemiology

Critical mass (infected cases) needed to trigger that?

Team and funding



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