HUMAN RHINOVIRUSES
Sneeze, wheeze and the ABCs

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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?
Usually..

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

**CLINICAL ENTITY**

**URT**
- Otitis media
- Rhinitis
- Coryza
- Pharyngitis
- Laryngitis

**LRT**
- Group [laryngo-tracheo-bronchitis]
  - Tracheobronchitis
  - Bronchitis
  - Bronchiolitis
  - Pneumonitis
Distinct human respiratory viruses

Human respiratory viruses
HRVs

- Human respiratory virus
- HRVs
Newly identified viruses (NIVs)

- Human respiratory viruses
- HRVs
- NIVs
HRV discovery

- Influenza virus 1933
- Coxsackie virus 1948
- Echovirus 1951
- Adenovirus 1953
- HRV 1953
- HRSV 1956
- HPIV 1956
- HCoV-229E 1966
- HCoV-OC43 1967

- HMPV 2001
- SARS-CoV 2003
- HCoV-NL63 2004
- HCoV-HKU1 2005
- HBoV 2005
- HRV-C 2006
- WUPyV 2007
History of the HRVs

1953
Improved cell culture

1956
First isolate
Type D.C. (HRV-9)

M & H viruses
Types JH/2060 (HRV-1)

1960
HRV-1 to HRV-55

1967
HRV-56 to HRV-89

1971
Asthma exacerbated by viral infection

1980
Major and minor groupings

1983
Antiviral groupings

1985
HRV-14 crystal structure
Canyon hypothesis

1990
Major receptor
is ICAM-1

1992
Minor receptor
is LDLR

1994
HRV A2 (C) 6 genomes

1996
40 HRV A and B genomes added

1999
All known HRV genomes sequenced

2002

2004

2006

2007

2009

2009

PCR

Enviroxime

Pirogavir

Rupintrivir

Pleconaril

BTA798

McErlean et al/
Renwick et al

Lamson et al/
Kistler et al

Aden et al/
Loons et al

Deffieres et al

Rothie et al
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)
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

Encode a single polyprotein
Co- and post-translationally proteolytically cleaved
4 capsid peptides (VP1, 2, 3, 4)
HRVs replicate in:
Nasal cells
Sinus cells
Bronchial epithelial cells
Smooth muscle cells

HRVs don’t replicate in:
Monocytes
Dendritic cells
~7kb ssRNA (+) genome
dsRNA intermediates

Small virion
~30nm
Non-enveloped
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
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

Acute Respiratory Illnesses (ARIs)

YEAR
- 2001
- 2002
- 2003
- 2004

% of all detections

Age of virus-positive individual

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 0-14-year 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

¹WHO GBD 2004/8; ²WHO Global Health Risks 2009; ³AIHW, 2003
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”

GAMA, 1988 — JOHNSTON/1993
WM LEE, 2007
STEININGER, 2001
BILLAUD/2003
GERNA/2006
DRUCE/1993
ANDEWEG, 1999
IRELAND/1999
LU/2008
TAPPAREL/2009
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

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

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

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
**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

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<th>A</th>
<th>B</th>
<th>C</th>
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<tbody>
<tr>
<td>1\textsuperscript{M,B}</td>
<td>34\textsuperscript{B}</td>
<td>C3 (f. QPM)</td>
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<td>C26</td>
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<td>97\textsuperscript{A}</td>
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Arden and Mackay. Virus Infections in Humans: Epidemiology and Control; 5th Edition  in press
~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?

1D (VP1)

HRV A
HRV-QPM 009
HRV-QPM 003
HRV-QPM 008
HRV-QPM 004
HRV-QPM 006
HRV-QPM 015
HRV-QPM 007
HRV-QPM 016
HRV-QPM 001*
HRV-QPM 002
HRV-QPM 011
HRV-QPM 012
HRV-QPM 017
HRV-QPM 013
HRV-QPM 005
HRV-QPM 010
HRV-QPM 014

HRV C
HRV-QPM >96% identity

HRV B
HEV

0.25 substitutions/site
Confirming the bimodal distribution
Single HRV types; highly specific RT-rtPCR

![Graph showing proportion of HRV-C type by month]

Proportion of HRV-C type by month

- **HRV-C6**
- **HRV-C3**
- **HRV-C10**

Month of collection

- Jan
- Feb
- Mar
- Apr
- May
- Jun
- Jul
- Aug
- Sep
- Oct
- Nov
- Dec

x 80
160 HRV types
# HRV-C3 (f.QPM) clinical impact

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

* Percentage of positives. \(^a\) HRV-QPM strain detected. \(^b\) Negative after all viral investigations (n=17). NC No chart available
Community Cohort
Victoria (Stephen Lambert and Terry Nolan)
2003, children followed or 12 months
Up to 6 HRV POS SRIs/yr

Hypothesis

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

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

36
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?