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Review

Human rhinoviruses: The cold wars resume

Ian M. Mackay [a,b,*]

[a] *Queensland Paediatric Infectious Diseases Laboratory, Sir Albert Sakzewski Virus Research Centre, Royal Children's Hospital, Queensland, Australia*

[b] *Clinical and Medical Virology Centre, University of Queensland, Queensland, Australia*

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Abstract

Background: Human rhinoviruses (HRVs) are the most common cause of viral illness worldwide but today, less than half the strains have been sequenced and only a handful examined structurally. This viral super-group, known for decades, has still to face the full force of a molecular biology onslaught. However, newly identified viruses (NIVs) including human metapneumovirus and bocavirus and emergent viruses including SARS-CoV have already been exhaustively scrutinized. The clinical impact of most respiratory NIVs is attributable to one or two major strains but there are 100+ distinct HRVs and, because we have never sought them independently, we must arbitrarily divide the literature's clinical impact findings among them. Early findings from infection studies and use of inefficient detection methods have shaped the way we think of 'common cold' viruses today.

Objectives: To review past HRV-related studies in order to put recent HRV discoveries into context.

Results: HRV infections result in undue antibiotic prescriptions, sizable healthcare-related expenditure and exacerbation of expiratory wheezing associated with hospital admission.

Conclusion: The finding of many divergent and previously unrecognized HRV strains has drawn attention and resources back to the most widespread and frequent infectious agent of humans; providing us the chance to seize the advantage in a decades-long cold war.

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Keywords: Rhinovirus; Review; New species; Diversity; Virus characterization; Respiratory virus

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* Correspondence address: SASVRC, Block C28, c/o Royal Children's Hospital, Herston Road, Herston, Queensland 4029, Australia. Tel.: +61 7 3636 8716; fax: +61 7 3636 1401.

E-mail address: ian.mackay@uq.edu.au.

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1. Introduction

'What had appeared to be a single disease capable of a single solution turns out to be something of unimagined complexity for which there is no straightforward answer.'
David Tyrrell and Michael Fielder. Cold Wars: The Fight Against The Common Cold, Oxford University Press, 2002.

Picornaviruses are the most common cause of viral illness worldwide (Rotbart and Hayden, 2000). Rhinoviruses (HRVs; previously called coryzaviruses, ECHO 28-rhinovirus-coryzaviruses (ERCs), muriviruses, enterovirus-like viruses, nasal secretion agents and Salisbury strains (Ketler et al., 1962; Jackson and Dowling, 1959; Andrewes, 1966; Hamre, 1967; Tyrrell and Parsons, 1960)) are the most common cause of acute respiratory tract illness (ARTI) and upper respiratory tract (URT) infections, traditionally defined as 'common colds'. ARTI is the leading cause of morbidity in children under 5 years (Bryce et al., 2005). No symptoms are pathognomonic for HRV infection (Phillips et al., 1968; Baer et al., 2007; van den Hoogen et al., 2003) so it devolves to the diagnostic laboratory to confirm the presence of HRVs (Barrett et al., 2006). Disappointingly, routine screening for HRV strains occurs infrequently because (i) testing is not always available (ii), HRV infection is often considered innocuous and (iii) because there are no treatments available. At the end of the 20th century, many aspects of HRV epidemiology, immunobiology, strain characterization and clinical impact remained poorly addressed (Couch, 1984; Monto et al., 1987). Nonetheless, HRVs are the most common reason for prescribing antibiotics to treat respiratory illness; even more so than bacterial infections (Rotbart and Hayden, 2000) and related costs exceed USD10 billion annually (Bertino, 2002). HRVs are also the most commonly identified pathogens associated with expiratory wheezing exacerbations, another burden on healthcare resources at many levels (Nyquist et al., 1998; Papadopoulos et al., 2002a; Jacques et al., 2006; Rotbart and Hayden, 2000; Monto, 2002; Jartti et al., 2004b).

HRVs are 'respiratory viruses', so-called because of their predominant detection in the human URT or occasionally in the lower respiratory tract (LRT). The circulation of respiratory viruses commonly varies over time and across distance, influenced by the number of strains and the strength and distinctiveness of their immunogenicity. The legion of antigenically distinct HRV strains has stymied the search for answers to epidemiology-related questions for decades (Andrewes, 1964) and as a result, HRVs are frequently dismissed as a single group of subtle variants.

Many past HRV studies were conducted without the advantages of modern molecular biology or knowledge of the many endemic respiratory viruses discovered since. With the application of the polymerase chain reaction (PCR) to HRV screening, it seemed possible to make great advances in our understanding of HRV infection; prior to this, detection of HRVs depended on subjective and insensitive methods ranging from deliberate human infection to cell or organ culture and serology. PCR is the best tool we currently have for better characterizing HRVs, but only when it is applied carefully and with a full understanding of its limitations (2007). Careful epidemiology plays a pivotal role in our understanding of the HRVs. Clinical follow-up of all HRV PCR positives, including in asymptomatic individuals or in control groups without the relevant respiratory symptoms, must occur hand-in-hand with regular and frequent sampling; an expensive undertaking.

In 2007, many divergent and previously uncharacterized HRV strains were identified (Arden et al., 2006; McErlean et al., 2007; Lamson et al., 2006; Kistler et al., 2007a; Lau et al., 2007; McErlean et al., 2008) using only molecular techniques. Herein we account for the molecular age by using 'strain' to describe the genetic equivalent of a serotype and 'variant' to describe viruses of the same strain (sharing 95–100% nucleotide identity) detected in different individuals. An 'isolate' is differentiated from a 'detection' by the inability to infer infectivity upon the latter; only isolates are propagated in culture.

New battles are underway against the most abundant and equally misunderstood viral foes humans have ever known. A fascinating but little understood battle in this war on colds is the one to quantify the contribution of HRV strains to illness and to modifying the naïve human immune system. This review will touch upon major areas of HRV research providing context for the latest surge in HRV discovery.

2. The human rhinovirus

A lack of response to antibiotic therapy, the absence of any microscopic bodies in inoculated cultures and the ineffectiveness of filtration to remove a causal agent suggested that a virus rather than a bacterium was the aetiological agent of the common cold (Mogabgab and Pelon, 1957). The first problem-plagued *in vitro* cultivation of an HRV, strain D.C., was reported in 1953 (Andrewes et al., 1953) but it was not properly characterized until 1968, by which time another variant had been given an official nomenclature (HRV-9) (Tyrrell, 1992; Kapikian et al., 1967; Tyrrell et al., 1962; Hamparian et al., 1961; Conant and Hamparian, 1968a). The HRVs were named for their association with morbidity involving the human nose and were eventually found to fill a causal role in respiratory illness, clinically distinguishing them from the genomically similar HEVs (Tyrrell et al., 1960; Taylor-Robinson, 1963).

2.1. Classification schemes: strains, types groups and species

By 1967, 55 distinct antigenic types or ‘serotypes’, had been recognized by the Collaborating Rhinovirus

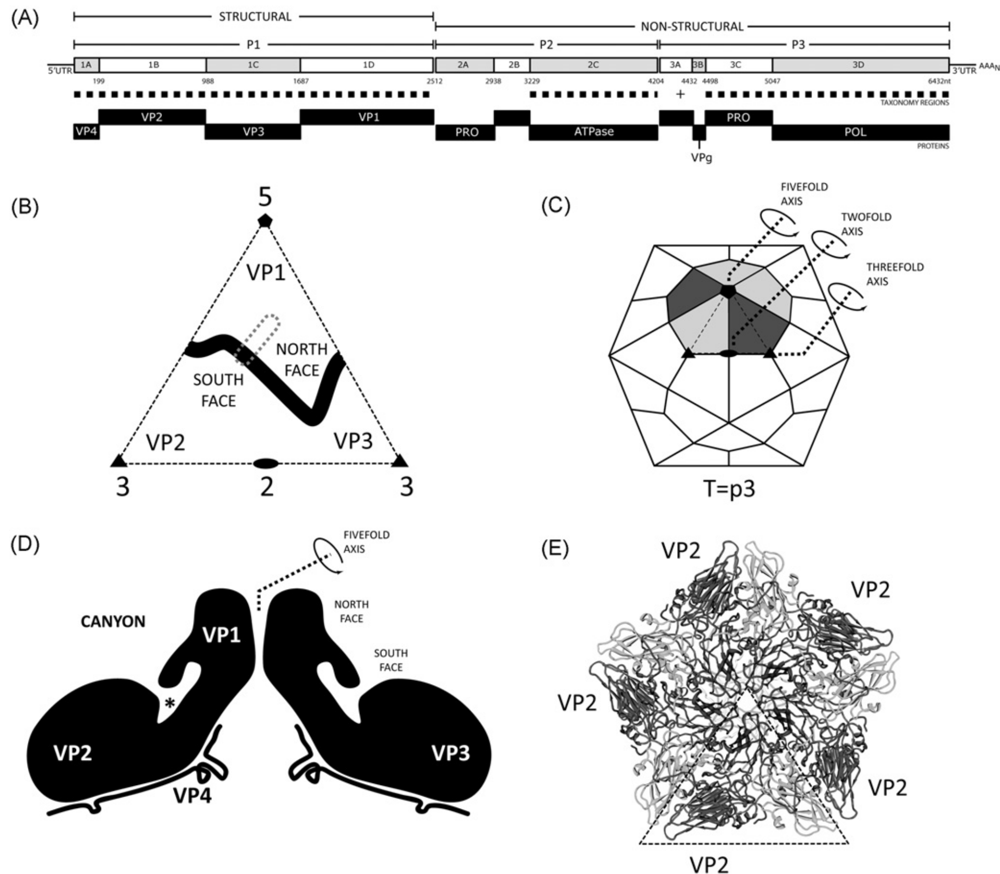


Fig. 1. Schematic representations of HRV components. (a) The genome structure (exemplified by HRV-QPM, GenBank accession number EF186077 including the nucleotide positions separating coding regions) is illustrated to identify the polyprotein and the subsequent precursory (P1-3) and matured proteins (named in filled boxes below the genome). The structural and non-structural regions encompass 11 proteins. Co-translational cleavage of the approximately 250 kDa polyprotein is mediated by the virus-encoded protease, $2A^{PRO}$ which catalyses its own release, freeing P1 from the P2 and P3 regions. All subsequent post-translational cleavages are performed by the 3C protease ($3C^{PRO}$), which is also likely to free itself from the polyprotein (Racaniello, 2001). The three regions commonly contributing to taxonomic placement are underlined by dashed bars. (b) A single asymmetric unit included to simplify the visualization of capsid features and symmetry. The approximate placement of the canyon (bold line; canyon faces also indicated) and the subsurface binding pocket (dashed grey oval) are indicated as are the axes of symmetry including a small filled pentagon indicating the 5-fold axis and a small oval and triangles indicating 2- and 3-fold axes of symmetry. (c) A depiction of an HRV icosahedral capsid comprising 60 of the smallest capsid units or protomers. A viral pentamer is highlighted (grey shaded panels) composed of five protomers. The HRVs assemble their capsid into an icosahedron. HRV virions are approximately 30 nm in diameter (Rotbart, 2002). (d) A pair of facing protomers (indicated on (c) by darker grey panels) depicted in cross-section using a space-filling format. Each protomer consists of a single copy of the three exposed structural proteins VP1-VP3 and the internalized structural protein, VP4 (7kDa) (Stirk and Thornton, 1994; Rossmann et al., 1985; Rotbart, 2002). The binding pocket (asterisk, ‘*’; usually filled by a ‘pocket factor’) is located beneath the ‘canyon’, a distinctive deep (2.5 nm) cleft; site of major receptor interactions. The canyon, comprised mostly of VP1 and VP3 residues, encircles the point at which five protomers come together to create the 5-fold axis (Rossmann, 1994; Rossmann et al., 1985). The canyon rim restricts entry of the 3.5 nm Fab portion of antibody molecules, evident in the conservation of the canyon floor which is sequestered away from immune pressures and provides a safe harbour for receptor contact among the majority of known HRV strains (Rossmann et al., 1985; Pevear et al., 1989). (e) Top view ribbon depiction of five protomers comprising a viral pentamer and their relative orientation. The asymmetric unit and VP2 are indicated for reference. Adapted from (Mackay et al., in press-a).

Laboratories, eventually increasing to 100+ (Dick et al., 1967; Kapikian et al., 1967; Hamparian et al., 1987). The family *Picornaviridae* (‘pico’ = Spanish for small, ‘rna’ = ribonucleic acid genome) currently comprises nine genera including *Enterovirus* and *Rhinovirus*. Within each of the two current species (*Human rhinovirus A*, 75 strains and *Human rhinovirus B*, 25 strains), HRV strains exhibit >70% amino acid identity and similar antiviral susceptibility patterns although recent studies indicate that some strains may not be distinct enough to deserve a unique name (Ledford et al., 2004). In 2004 a proposal was set before the International Committee on Taxonomy of Viruses (ICTV) to merge the genera *Rhinovirus* and *Enterovirus* into a single genus, *Enterovirus*, with species remaining intact and under the banner of a new virus order, *Picornavirales* (Le Gall et al., 2008). The issue of merging the genera has been one of contention for decades (Rosen, 1965; Skern et al., 1985; Stanway et al., 1984).

HRVs have been subclassified using tissue tropism and host range, antiviral susceptibility and phylogeny. Early sequencing of subgenomic RT-PCR amplicons also identified the presence of two viral clades, subsequently defined as the HRV species (Mori and Clewley, 1994), a distinctive bifurcation retained when sequences from other subgenomic regions, including P1, 2C and 3CD (Fig. 1) are compared. Today, sequencing and phylogenetic comparison is a surrogate for all the important biological classification criteria (Savolainen et al., 2002, 2004; Ledford et al., 2004; Laine et al., 2005, 2006; King et al., 2000; Hungnes et al., 2000) (Table 1).

2.2. The rhinovirion

The HRV capsid contains an approximately 7.4 kb RNA genome (Fig. 1a), which tends to be adenine and uracil (A + U) rich (Hughes et al., 1989), particularly in the third or ‘wobble’ codon position. The coding region is bracketed by untranslated regions (UTRs) which perform regulatory functions that permit genome duplication and production of a single, multi-domain, proteolytically processed ‘polyprotein’ (Rueckert and Wimmer, 1984).

The mature capsid proteins VP1 (34–36 kDa), VP2 (27–30 kDa) and VP3 (24–28 kDa) all exist as a convoluted set of protein sheets and loops (Stirk and Thornton, 1994; Ledford et al., 2005) (Fig. 2). The loops protrude beyond the external capsid surface and contain important, often discontinuous, antigenic sites. Four neutralizing antibody immunogenic (NIm) regions have been identified on the HRV-14 and HRV-16 virion; NIm-1A (located in VP1), NIm-1B (VP1), NIm-II (VP2 and VP1) and NImIII (VP3 and VP1) (Rossmann et al., 1985). Antigenic sites (A, B and C) have also been identified on HRV-2, a minor group virus (Rossmann et al., 2002).

Picornaviruses recognize a variety of receptors (Rossmann, 1994; Khan et al., 2008; Uncapher et al., 1991). The capsid of the majority of HRVs (n = 89; described as the ‘major’ group (Abraham and Colonno, 1984)) interacts with the amino-terminal domain of the 90 kDa intercellular adhesion molecule (ICAM-I; CD54 (Greve et al., 1989; Staunton et al., 1989; Rossmann et al., 2000; Tomassini et al., 1989). Receptor binding destabilizes the HRV capsid, probably by dislodging the ‘pocket factor’ and initiating uncoating (Rossmann et al., 2002; Kolatkar et al., 1999; Rossmann, 1994).

The remaining minor group (Abraham and Colonno, 1984) of viruses employ members of the low density lipoprotein receptor (LDLR) family to attach to cells (Hofer et al., 1994). Binding of VLDL-R occurs outside of the canyon’s

Table 1
Summary of historical and current HRV groupings

	Human rhinovirus A antiviral group B		Human rhinovirus B antiviral group A
1^{M}	**34^{H}**	**64^{H}**	**3^{H}**
2^{M}	**36**	**65**	**4**
7^{H}	**38^{H}**	**66**	**5**
8^{H}	**39**	**67**	**6^{H}**
9^{H}	**40^{H}**	**68**	**14^{H}**
10^{H}	**41**	**71**	**17^{H}**
11^{H}	**43**	**73**	**26^{H}**
12^{H}	44	**74**	**27^{H}**
13^{H}	**45**	**75**	**35**
15^{H}	**46**	**76**	**37**
16^{H}	47	**77**	**42**
18^{H}	49^{H}	**78**	**48**
19^{H}	**50**	**80**	**52**
20^{H}	**51^{H}**	**81**	**69**
21^{H}	**53**	**82**	**70**
22^{H}	**54**	**85**	**72**
23^{H}	**55**	**87**	**79**
24^{H}	**56**	**88**	**83**
25^{H}	**57**	**89**	**84**
28^{H}	**58**	**90**	**86**
29^{M}	**59**	**94**	**91**
30^{M}	**60**	**95**	**92**
31^{M}	**61**	**96**	**93**
32	62	**98**	**97**
33^{H}	**63**	**100**	**99**
		HANKS	

Minor group HRV strains are underlined, major group are shown in bold. M (only found in HRV A; propensity for monkey cells) and H strains (located in both species; grew in human cell cultures) (Gwaltney and Jordan, 1964; Stott et al., 1969; Rosenbaum et al., 1971; Tyrrell and Parsons, 1960; Tyrrell and Bynoe, 1961; Ketler et al., 1962; Taylor-Robinson and Tyrrell, 1962; Cooney and Kenny, 1977; Bloom et al., 1963) are indicated with superscripts and generally correlate with receptor usage (Macnaughton, 1982). Assignment of some strains to this sub-classification may also have been influenced by the viral load in the inoculum (Douglas et al., 1966a; Hamre, 1967). The M and H terminology was abandoned in favour of a more streamlined sequential numbering system (Taylor-Robinson and Tyrrell, 1962). HRV strains were later divided into the major and minor groups defined by receptor tropism (Abraham and Colonno, 1984; Colonno et al., 1986). Two ‘antiviral groups’ (A and B) were defined by their susceptibility to antiviral molecules, reflected by the amino acid sequence of the interacting regions (Andries et al., 1990). HRV-87 is included in this table although it is a variant of HEV-68 (Ishiko et al., 2002), despite its apparent sensitivity to acid (Uncapher et al., 1991). HRV-Hanks and HRV-21 may be the same serotype as may HRV-8 and HRV-95 (Ledford et al., 2004).

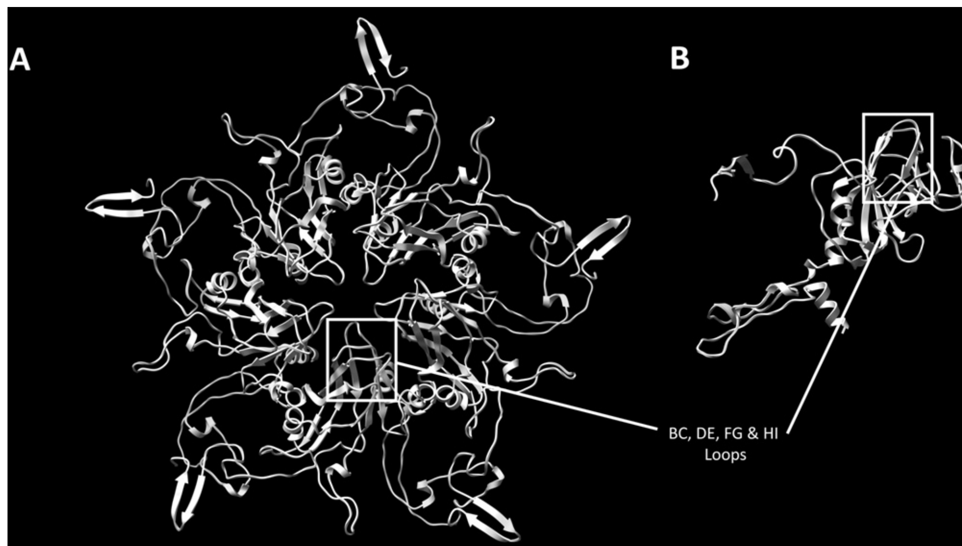


Fig. 2. A ribbon depiction of the VP1 proteins involved in the predicted HRV-QPM pentamer (constructed using Chimera (Pettersen et al., 2004); derived from (McErlean et al., 2008)) viewed (A), from above and (B), as a single VP1 molecule from the side. Significant surface-exposed loops which contain important antigenic sites are boxed. β-sheets, flat ribbons with arrowheads; α-helices, coiled ribbons.

Northern face (Fig. 1) employing a different destabilzation mechanism for virus uncoating.

3. HRV strains: more than meets the eye?

HRV strains are usually presented in the literature as a tight-knit group of viruses with identical clinical impact but there is some evidence to suggest closer scrutiny of individual strains is warranted.

3.1. Host immunity: raising the iron curtain

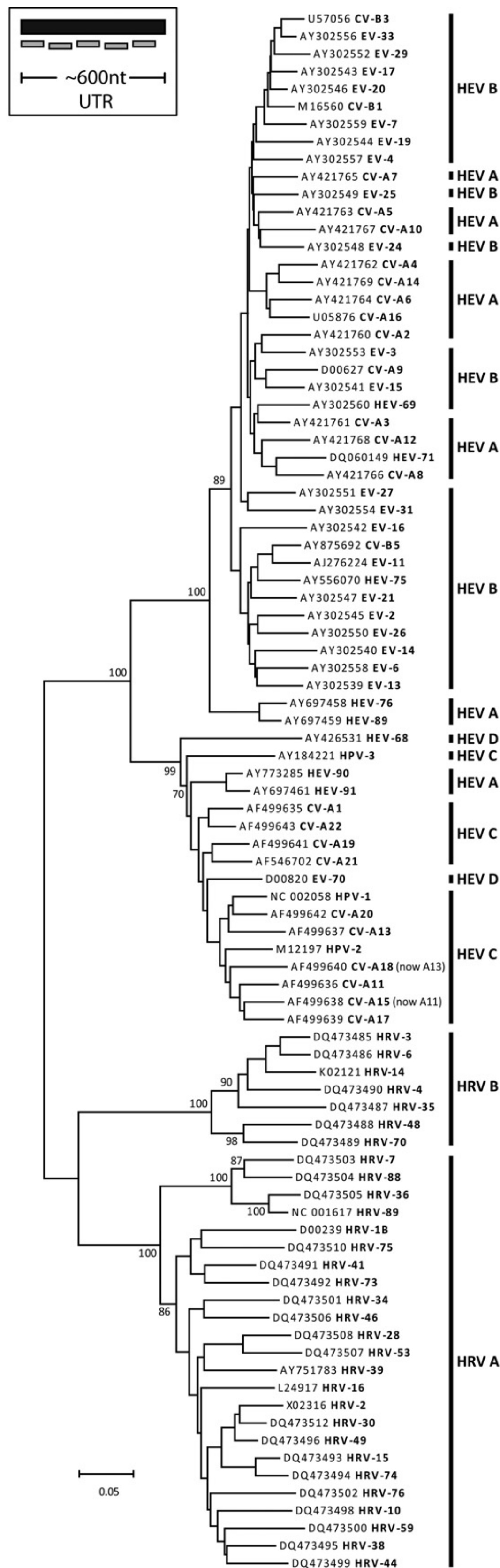
HRV strains seem to circulate without any identifiable pattern (Phillips et al., 1968). However, the apparent randomness can today be explained by the complex interaction between the many strains, host genetic heterogeneity and the unique immune repertoire accrued by each person and population over a lifetime of rhinoviral exposures. Each new infection contributes to the accumulation of immunity which decreases symptom severity upon reinfection by, or affords complete protection from challenge with, an homologous strain (Cate et al., 1964; Bloom et al., 1963); host immunity 'perceives' strains as antigenically distinct (Douglas et al., 1966b; Dick et al., 1967). Immunogenic novelty is is also supported by the occurrence of sequential infections by different HRV strains (Peltola et al., 2008).

3.2. Sequence conservation: time may not weary them

In 2007 it was suggested that, in contrast to the HEVs, evolution of HRV strains was not subject to significant recombination (Kistler et al., 2007b) despite the opportunity provided by the occurrence of dual infections (Cooney and Kenny, 1977). In fact, HRV strains were suggested to be under 'purifying' pressure driving minimal change over time, apart from variation at sites of immune activity. Recently, an HRV has been described which may have resulted from a recombination event between the P3 region of two HRV A strains (Tapparel et al., 2008). Nonetheless, the 5'UTR from HRV strains identified decades earlier, retains the same conserved sections (Andeweg et al., 1999), making it a good region for molecular typing of HRV strains (Lee et al., 2007). In our hands, it has proven less useful for clearly assigning HEV strains to their current species (Fig. 3). In Brisbane, we have identified contemporary variants of HRV strains first classified in 1967, that retained 100% amino acid and >90% nucleotide identity with their elder namesakes (1B region; data derived from Arden et al. (2006)).

3.3. Clinical outcome: too early to dismiss strain-specific clinical features?

There are no data that convincingly identify a distinct clinical outcome for any single HRV strain. Many studies have detected some strains more or less frequently (Table 2) but they do not seek all likely microbial causes or even span sufficient time to encompass all possible HRV strains. Andries et al. suggested that antiviral group B strains (HRV A) were over-represented among symptomatic respiratory infections (SRIs) ascribing a more frequent role in disease to these strains but this could also be attributed to the greater number of strains in this group. A more practical finding was that less HRV-39 (HRV A) than HRV-14 (HRV B) was required to infect antibody-free adult volunteers, the latter also producing colds of milder severity (Hendley et al., 1972). In 2006, a birth cohort study noted that infants who wheezed with infection by one HRV strain did not always wheeze with a second



or third HRV infection (Kusel et al., 2006) suggesting some strains may be more 'asthmagenic' (Yoo et al., 2007).

4. Forty years of rhinovirus detection

Poor detection of the HRV strains has long been a hindrance to their characterization. Culture has proven unreliable (Kaiser et al., 2006; Larson et al., 1980; Jartti et al., 2004b) and identification of a large number of divergent HRV strains in 2007 suggested that prior investigations detected only some strains and not others. The extent of this bias may have contributed to a significant underestimation of the number of strains and the capacity of HRVs to cause illness. The respiratory virus diagnostic tool *de jour* is PCR but positive results are seldom characterized beyond the genus level and are usually reported as 'respiratory picornaviruses' (HRVs and HEVs detected in the respiratory tract).

Serodiagnosis is impractical (Johnston et al., 1993a; Gwaltney, 1966) but serological techniques could have a role in gauging the extent to which single HRV strains undergo antigenic change from year to year. Nonetheless, antibodies are essential for strain-specific neutralization of infection (Ketler et al., 1962), techniques around which the HRV nomenclature system evolved (Conant and Hamparian, 1968b). Such techniques have found that a large number of distinct strains circulate each year and that a selection of them predominate in a given season, replaced by others in subsequent years (Andrewes, 1966; Gwaltney and Hendley, 1978).

4.1. Cell culture methods

In 1953 Andrewes and co-workers at the Common Cold Unit (Salisbury, UK) described the first isolation of an HRV strain using cells from a particular human embryo, but propagation failed once this source of cells was exhausted (Andrewes et al., 1953; Andrewes, 1966). Early *in vitro* isolation methods employed an interference test to determine successful isolation; cultures suspected of infection with an HRV prevented infection by another, readily titratable virus (Andrewes, 1966). Later, Price (1956; the JH strain) and then Pelon et al. (1957; 2060 strain) developed improved culture systems that permitted viral replication to be more easily identified (Pelon et al., 1957; Price, 1956). These strains were initially classified as echoviruses (ECHO 28; later HRV-1) (Pelon, 1961). At the same time, propagation of the HGP

Fig. 3. Phylogeny of the untranslated regions (UTR; 5′ 610–635 nt) of HRV and HEV sequences obtained from GenBank (accession numbers shown). For this review, the region was also divided into 100 and 200 nt fragments and trees were constructed and compared for each consecutive fragment (inset; grey bars represent 100 nt fragments; black bar represents complete UTR). The branching patterns shown were retained with only subtle changes; HRV species were always represented clearly, as shown. HEV species were muddled. Nucleotide sequences were aligned using BioEdit v7.0.5.3 (Hall, 1999) and trees were constructed from a Neighbor-Joining analysis with 500 resamplings using Mega v4.0 (Tamura et al., 2007). Nodal values were shown only until they fell below 70. HEV, human enterovirus; EV, echovirus; CV, coxsackievirus; HPV, human poliovirus.

Table 2
Examples of HRV strains described in studies of specific clinical outcomes

Study category	Clinical presentation or outcome	Predominating HRV strains	Year published
Adult (Family, Student) (Dick et al., 1967)	URT illness	43, 55	1967
Adult (Student) (Phillips et al., 1968)	URT illness	7,9,13,14,17,56,64	1968
Children (Read et al., 1997)	Recurrent asthma	13,29,32,48,49,81	1997
Adult (Military) (Bloom et al., 1963)	URT illness	1A, 1B, 2	1963
Adult (Outpatients) (Suzuki et al., 2007)	PVOD	40,75,78,80	2007
Adult (Military) (Rosenbaum et al., 1971)	Common cold	1A, 2, 38, 7, 64, SD-7407	1971
Adult (Office workers) (Gwaltney et al., 1966)	ARTI	4,9,10,12–14,16,24,35,39	1966
Adult (Lung transplant) (Kaiser et al., 2006)	Graft dysfunction	3,27,64	2006
Adult (LTCF) (Louie et al., 2005)	LRT illness	82	2005
Adult (Surgery) (Craighead et al., 1969)	LRT illness	13	1969
Post-mortem (infants) (Urquhart and Grist, 1972; Urquhart and Stott, 1970	SIDS	15, 22	1972,1970
Adult (Isolation) (Holmes et al., 1976)	LRT illness	2	1976
Children (isolation) (Stott et al., 1969)	LRT illness	1B,10,16,18,19,32,33,38,40,49,51,65,78	1979
	URT illness	12,23,50	
Children (Krilov et al., 1986)	LRT illness	1B,44,53,56,63,88	1986
Children Adults (Reilly et al., 1962)	ARTI-Ab^{NEG} for common viruses	10,12,13,18,19,23–25,27,28 7–12,19–22,58	1962

Ab, antibody; ARTI, acute respiratory tract infection; ILI, influenza-like illness; LRT, lower respiratory tract; LTCF, long term care facility; PVOD, post-viral olfactory disease; SD-7407, untyped HRV strain; SIDS, sudden infant death syndrome; URT, upper respiratory tract.

(HRV-2) strain resulted from using increased acidity, lowered cultivation temperatures and constant motion (rotation) (Tyrrell et al., 1960; Parsons and Tyrrell, 1961). Despite the challenges (Mogabgab and Pelon, 1957), virus isolation is a more sensitive indicator of infection than an antibody rise in paired sera (Hendley et al., 1972).

Several cell lines and methods are required to encompass virus concentrations that range from 10^1 to 10^5 $TCID_{50}$/mL (Douglas et al., 1966b; D'Alessio et al., 1984; Cate et al., 1965; Hendley et al., 1973). Additionally, controlling cell age after plating (<72 h), inoculum volume, medium pH (6.8–7.3) and cell density is important for the reproducible appearance of HRV-induced plaques and for higher virus yields (Sethi, 1978; Gwaltney, 1966; Behbehani and Lee, 1964; Fiala and Kenny, 1966). The HRVs can grow at temperatures above 35 °C but rolling at 33 °C, preceded by a 2–4 h stationary incubation period (Parsons and Tyrrell, 1961) produces the highest yield and fastest *in vitro* viral growth (Papadopoulos et al., 1999b; Rosenbaum et al., 1971; Andrewes, 1966; Behbehani and Lee, 1964).

4.2. Polymerase chain reaction: weapon of choice for rhinovirus detection

The improved sensitivity of PCR-based assays dramatically increased the frequency of HRV detection compared to cultivation methods (Pitkäranta et al., 1997; Vesa et al., 2001; Kämmerer et al., 1994; Andeweg et al., 1999; Arruda et al., 1997; Renwick et al., 2007). It is becoming commonplace to find HRVs as the predominant virus in ARTI cases (Aberle et al., 2005; Versteegh et al., 2005; Hutchinson et al., 2007). No PCR assays have been completely validated against all picornavirus strains using clinical material, a pedantic but nonetheless necessary process, yet many successfully detect the currently circulating HRV strains at levels as low as 10^2 $TCID_{50}$/sample. This amount is commonly shed during experimental inoculation studies (Arruda and Hayden, 1993; Lu et al., 2008). Because HRV strains are being detected beyond their commonly understood symptomatic context (Johnston et al., 1993b; Suvilehto et al., 2006) it is important to define a qualitative or quantitative correlation between viral nucleic acid detection and the presence of infectious virus at the sampling site, a problem when using PCR to study respiratory viruses (Mackay et al., 2007). Today, the most commonly employed oligonucleotides comprising the 'Gama assay' (OL26 and OL27, (Gama et al., 1988)), remain at the forefront of detecting respiratory picornaviruses (Torgersen et al., 1989; Gern et al., 2000; Spence et al., 2007; Winther et al., 2007; Arruda and Hayden, 1993; Xatzipsalti et al., 2005; Papadopoulos et al., 1999a,b; Khetsuriani et al., 2007; Arruda et al., 1997; Pitkäranta et al., 1997, 1998; Johnston et al., 1993b; Blomqvist et al., 1999; Nokso-Koivisto et al., 2002; Kusel et al., 2006; Seemungal et al., 2001). However, subtle repositioning of these primers or changes to the method of employing them (Ireland et al., 1993; Collinson et al., 1996; Deffernez et al., 2004; Jartti et al., 2004b) may be detrimental to assay performance, suggested by predicted hybridisation mismatches and unusually reduced detection frequencies. Other oligonucleotides used in HRV studies include (Hayden et al., 2003; Wright et al., 2007; Steininger et al., 2001; Billaud et al., 2003; Hyypiä et al., 1989; Freymuth et al., 2000; Halonen et al., 1995; Blomqvist et al., 1999, 2002; Vesa et al., 2001; Coiras et al., 2004). In common with the Gama assay, some diagnostic oligonucleotides used for HRV PCR-based studies share identity with HEV sequences and vice versa (Leparc et al., 1994; Lina et al., 1996). In our hands, the Lu assay (Lu et al., 2008) functions as a suitable real-time PCR alternative and is useful for epidemiology studies seek-

ing to better represent respiratory picornavirus prevalence. The use of oligoprobes increases amplicon detection sensitivity and specificity, identifying 100-fold fewer $TCID_{50}$/mL or 10-fold fewer genome copies, than agarose gel detection of amplicon (Johnston et al., 1993b; Andréoletti et al., 2000; Lu et al., 2008).

When they are included in the testing menu, HRV detections can raise the frequency of pathogen detection above one per sample (Brunstein et al., 2008). Early HRV studies found that HRV strains were frequent contributors to co-infections and concluded this was evidence for a minor role in serious respiratory illness (Stott et al., 1969). More likely this reflected the insensitivity of the culture methods used which simply failed to propagate many HRV strains. Today, half of all HRV detections can be found concurrently with another virus, on the surface, a significant fraction, and yet 80% or more of human respiratory syncytial virus (HRSV), human metapneumovirus (HMPV), HEV and influenzavirus (IFV) detections and 71% of human coronavirus (HCoV)-NL63 detections can be found in the company of another virus (Richard et al., 2008). Considering their ubiquity, it is interesting that relatively low numbers of concurrent detections of other respiratory viruses occur with HRV strains (Mackay, 2007; Lambert et al., 2007), supporting the concept that HRVs have a direct role in the clinical outcome of their infection (Miller et al., 2007). In fact, HRV strains are co-detected with other pathogens in reproducible, but clinically undefined, patterns (Brunstein et al., 2008). The HRV partnership with host immunity may be a mutualistic one, inadvertently imparting an advantage to the host by protecting against more cytopathic respiratory viral pathogens while the host provides a vessel for HRV replication and transmission.

Novel multitarget molecular laboratory tools include the MultiCode-PLx system which employs a synthetic nucleobase pair, multiplex PCR and microsphere flow cytometry (Nolte et al., 2007) and permits the discrete detection of 17 viral targets and two assay controls although returning an untypically low HRV detection rate. Similar technology also provides a sensitive, 20-target, 2-step RT-PCR-based assay (Mahony et al., 2007). A novel approach to amplicon detection entitled 'MassTag' (USD10-15 per sample) can discriminate 20 viral PCR products (Briese et al., 2005) using oligonucleotides tagged with a unique compound that is released via a photolabile link (QIAGEN). The MassTag approach has successfully identified novel HRV sequences (Renwick et al., 2007; Lamson et al., 2006). Microarrays can detect thousands of viral targets (USD30-300 per sample) but are not sensitive enough to avoid a pre-hybridisation PCR amplification when using clinical specimens. At their most robust, microarrays, like PCR, rely on the existence of conserved regions of sequence to detect unknown viruses and they have succeeded in detecting previously unknown HRV strains (Wang et al., 2002). Rapid protein- or virion-based assays are not (yet) adequately sensitive (Ostroff et al., 2001; Shanmukh et al., 2006).

5. Rhinovirus circulation patterns

HRV detections occur throughout the year but usually peak in spring and autumn (Winther et al., 2006; Vesa et al., 2001; Silva et al., 2007; Miller et al., 2007; Gwaltney et al., 1966; Fox et al., 1975; Lambert et al., 2007; Jartti et al., 2004b) depending on the detection tools employed, the length of the study period and the type of population investigated (Phillips et al., 1968; Wald et al., 1995). One study indicated that any given strain may have a 'lead time', providing warning of an impending epidemic by that strain (Dick et al., 1967) and identifying another purpose for strain typing. Few data address whether every strain recurs each year at a single location, if herd immunity protects against reinfection by the previous epidemic strain or for how long such an effect might last. We found in Brisbane that during 2003, HRV B strains circulated during winter whereas the HRV As occurred in all seasons; a novel HRV clade, HRV A2, predominantly circulated during spring (Arden et al., 2006). Return from long school or university holidays is a frequent trigger for HRV epidemic activity in the young (Johnson et al., 1964; Al-Sunaidi et al., 2007; Hamre et al., 1966; Johnston et al., 2006). Seasonal variation in the prevalence of any virus may be influenced by interference, whereby the peak prevalence of one respiratory virus moderates or prevents the processes that let one or more other viruses establish themselves at the same time (Glezen and Denny, 1973).

The reported rate of HRV infection (Table 3) varies with the manner in which illnesses are defined, recorded, documented aetiologically, and tracked longitudinally (Lemanske et al., 2006). But detection rate data do not comprehensively represent HRV circulation patterns because sequential infections by different strains occur and may appear as unbroken symptomatic episodes during a single observation period (Fig. 4) (Phillips et al., 1968; Minor et al., 1974c); an occurrence which is rarely examined. In other instances, multiple HRV strains can be isolated (Cooney and Kenny, 1977; Cooney et al., 1972) or detected (Renwick et al., 2007; Lee et al., 2007; Peltola et al., 2008) from a single specimen indicating a capacity for HRV co-infection.

5.1. Rhinovirus infection: occupation or skirmish?

HRV shedding is commonly limited to a 10–14 day period in immunocompetent subjects, although shedding or illness may not be detectable even after experimental inoculation (Kaiser et al., 2006; Douglas et al., 1966b; Rosenbaum et al., 1971; Suzuki et al., 2007; Dick et al., 1967; Arruda et al., 1997). Adults and children are usually asymptomatic by the 5th day post-infection (Cate et al., 1964). Chronic carriage does not commonly occur in children but the detection of picornavirus RNA by RT-PCR reportedly extends into the 2nd or 3rd week after onset, by which time the child has been defined as asymptomatic for a week or more (Winther et al., 2006). As shown in Table 4, HRVs are not alone in their detection from asymptomatic populations, yet they seem to be

Table 3
Examples of the rate of respiratory virus infection or illness among different populations

Study category (virus/symptoms)	Rate	Detection method
Students (HRV) (Phillips et al., 1968)	1.6 per 9 months	Culture
Adults (military) (Rosenbaum et al., 1971)	40% ≥2 per month 10% ≥3 per month	Culture
Infants (any virus) (Lemanske et al., 2006)	7.7 per 12 months 5.8 mild, 1.9 severe	PCR
Children (any microorganism) (Collinson et al., 1996)	<6 years, 3.4 per 12 months >6 years, 2.3 per 12 months	PCR
Children (PV) (Winther et al., 2006)	0.51 per child-month	PCR
Children (HRV) (Miller et al., 2007)	4 per 1000 per year	PCR
Isolated community (common cold) (Paul and Freese, 1933)	1 per year	Bacterial culture and Observation
Isolated community (common cold) (Milam and Smillie, 1931)	0.8 per year	Bacterial culture and Observation
Home care (Wald et al., 1988)	4.7 per year	Observation
Group care (Wald et al., 1988)	6.0 per year	
Day care (Wald et al., 1988)	7.1 per year	

HRV, human rhinovirus; PCR, polymerase chain reaction; PV, picornavirus.

over-represented amongst claims that such detections make a virus an innocuous passenger in the host.

Isolation of the same HRV strain on two or more discrete occasions has been reported among military recruits, one strain being isolated intermittently over a 4-week period (Rosenbaum et al., 1971). Among healthy adults, this length of excretion is significant, however cultivation failure from intermediate samples with lower viral loads and heterotypic cross-reactivity which influences the strain typing system cannot be excluded. Viral RNA detection, usually conducted in the absence of any strain typing, has been suggested to occur days before to five or more weeks after symptoms commence or cease, respectively (Winther et al., 2006; Jartti et al., 2004a; Pitkäranta et al., 2005). The implication is that samples taken only during an SRI could be HRV-negative while sampling outside this period may detect the virus in the absence of symptoms (Winther et al., 2006). Unfortunately, few data are available to address this since clinical follow-up is rare (Suvilehto et al., 2006). When parents maintained a daily symptom diary, 81% of respiratory picornvirus RT-PCR

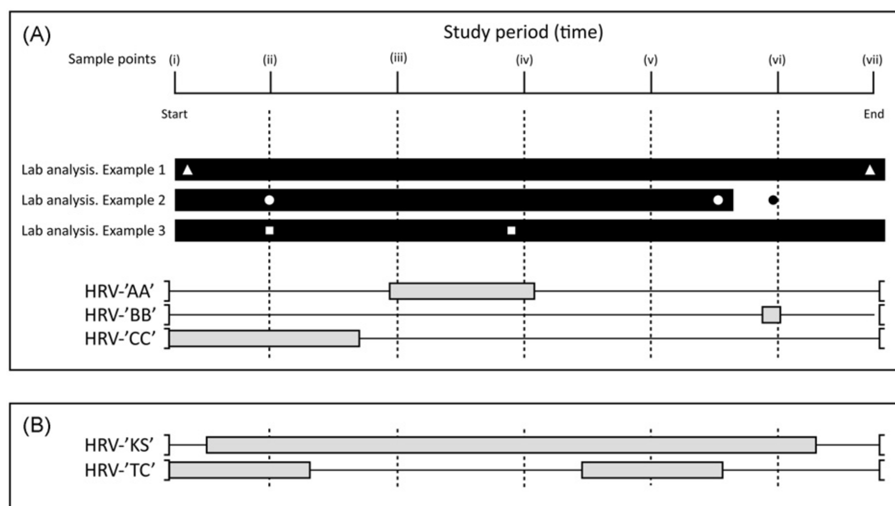


Fig. 4. The importance of sampling time and strain typing when investigating HRV shedding. (A) The examples provided here represent different, hypothetical studies for a single individual. If sampling occurred at each time point (i–vii) and if each positive was characterized, three different HRV strains could be identified from a single subject (period of replication by each strain indicated by a grey box; strain identity is provided on the left). If typing was not performed and sampling only occurred intermittently (indicated by the shapes in Example 1 (triangles), 2 (circles) and 3 (squares)), then the laboratory data could suggest only one or two infections. If testing was only performed during a symptomatic period (usually when the study starts) and again at the conclusion of the study period (Example 1), accurate determination of HRV infection frequency or strain diversity would not be possible. Example 2 identifies a case when sampling at the cessation of a study would not identify the HRV-BB strain however symptoms could be apparent due to its soon-to-be-detectable (detection indicated by the filled circle) replication. Strain BB may be prohibited from significant replication (indicated by the brevity of the infection period) due to pre-existing immunity which results in an asymptomatic state that still yields a relevant positive detection. In Example 3, sampling is only conducted for an SRI and so HRV BB is not detected. (B) HRV-KS is a truly chronic infection by a single strain whereas HRV-TC exemplifies some culture-based studies where the same strain appeared to recur after a period of absence, in the same individual. Some of these study designs could conclude persistent shedding was occurring, confounding attempts to correlate symptomatic periods with individual HRV strains and perpetuating the belief that HRVs do not exist independently of their group.

Table 4
Examples of studies detecting a respiratory picornavirus in asymptomatic or control groups

Study category (detection method; virus)	Other viruses detected in group	Frequency (%)
Birth cohort (PCR; PV) (Kusel et al., 2006)	HCoVs, HPIVs, HMPV, HRSV, HAdVs	2
Children/adults (PCR; PV) (Johnston et al., 1993b)	ND	12/4
Children (PCR; PV) (Winther et al., 2006)	ND	19
Adult (culture; HRV (Gwaltney et al., 1966)	IFVs, HPIVs, CVA-21, HRSV	2
Adult (culture; filterable agent) (Jackson and Dowling, 1959)	ND	10
Children (PCR; HRV) (Suvilehto et al., 2006)	ND	61
Children (culture; HRV) (Banham, 1965)	HPIV, HEVs, βHS, HAdVs	2
Adults (culture) (Hamre et al., 1966)	HCoV, HRSV, FLUBV, HPIVs, HAdVs	≤5
Children <2 years/2–16 years (PCR; HRV) (Rakes et al., 1999)	HRSV, HCoV-OC43, HAdVs	41/36
Children (PCR; PV) (Jartti et al., 2004a)	Not examined.	10
Children (culture) (Stott et al., 1969)	HRSV, HRV, HPIV, HSV, HAdV, HEV	9

ND, not described; PV, respiratory picornavirus; βHS, β-haemolytic streptococci; FLUBV, influenza B virus; HAdVs, human adenoviruses; HCoV, human coronavirus; HMPV, human metapneumovirus; HPIV, human parainfluenzavirus; HRSV, human respiratory syncytial virus; HSV, Herpes simplex virus; IFV, influenza viruses not otherwise typed.

positives were associated with illness while 19% were not, however ‘asymptomatic’ periods may also have been associated with underreporting of true SRIs (Winther et al., 2006). One concludes that it is essential to sample often for accurate HRV epidemiology.

Epidemiology employing PCR together with molecular genotyping usually does not identify chronic shedding (Peltola et al., 2008) and it is more likely the occurrence of serial or concurrent infections by multiple strains (Dick et al., 1967; Rosenbaum et al., 1971). One must therefore be cautious when describing persistent HRV infections (Kling et al., 2005) in the absence of strain typing investigations if the implication is that persistence is a feature of individual HRV strains. Virus shedding normally ceases within days (Gern et al., 2000). It is likely that any plan to link HRV positivity with asymptomatic carriage in otherwise healthy individuals will require carefully constructed aims, regular and frequent sampling and a clinical scoring system capable of determining a real asymptomatic state. The current data indicate that truly chronic shedding (>8 months) of a single HRV strain is limited to those individuals with underlying immunosuppression or immune dysfunction (Kaiser et al., 2006).

6. Rhinovirus transmission

The nasopharynx has been identified as the major site of focal virus production, regardless of experimental inoculation route (Winther et al., 1986) therefore much of the data identifying specimen types and transmission routes have arisen from studies of URT illness. Point inoculation of the conjunctival sac, in the absence of neutralizing antibody (≤1:2), results in active infection of the nasal mucosa after virus is carried through the nasolacrimal duct (Winther et al., 1986). In these and other experimental inoculation studies virus is commonly delivered by aerosol or intranasal instillation of 0.25–5 mL of virus suspension (Douglas et al., 1966b; Winther et al., 1986; Hendley et al., 1972; Turner et al., 1998; D’Alessio et al., 1984; Cate et al., 1965; Mallia et al., 2006). Nasopharyngeal washes yield better viral isolation rates than either nasal or pharyngeal swabs, but the convenience of swabs has meant their use is more widespread (Cate et al., 1964; Gwaltney et al., 1966; Peltola et al., 2008) especially now that the presence of viable HRV is not necessary for virus identification.

The high frequency of clinical colds and their low transmissibility was once thought enigmatic (Andrewes, 1964, 1966). This feature of early HRV epidemiology has been largely demystified today with our current knowledge of 100+ strains, a grasp of the immunology involved, the identification of asymptomatic infections and a vastly improved diagnostic sensitivity. In the past, household cross-infection, determined by SRI, was low; about five exposures to infected members required for infection (Lidwell and Sommerville, 1951) despite viral loads in nasal washings peaking at 1.6×10^5 $TCID_{50}$/mL (Douglas et al., 1966b). Experimental transmission was also reportedly inefficient (D’Alessio et al., 1984). In contrast, ‘naturally’ close-quartered military populations, interacting over 1–4 weeks experienced rapid spread of HRV strains to >50% of the group (Rosenbaum et al., 1971). The use of PCR recently clarified this discrepancy confirming that frequent transmission in families is more common than culture-based studies identified, often resulting in asymptomatic infection among older siblings and parents (Peltola et al., 2008).

In the laboratory, HRVs can retain infectivity for hours to days on suitable surfaces (Hendley et al., 1973) supporting the practical possibility of self-inoculation via fomites (Hendley and Gwaltney, 1988). Hand-washing and disinfectant wipes are likely methods of interrupting transfer from fomites to the nose or to conjunctivae (Gwaltney et al., 1978; Hendley and Gwaltney, 1988; Winther et al., 2007). However, eye-rubbing and nose-picking occur frequently (Hendley et al., 1973), suggesting inoculation could outpace personal hygiene.

Transmission of HRVs by large- and small-particle aerosol has generally proven inefficient, supported by a low isolation rate from saliva (39% compared to 65% of hand washes and 50% of nasal swabs) (Gwaltney et al., 1978; Douglas et al., 1966b; Hendley et al., 1973) and from only 8.3% of participants exposed to large particle aerosols (Gwaltney et al.,

1978). In fact, wild HRV strains induce colds that spread less effectively than those due to coxsackievirus A 21 infection (Couch et al., 1970; Gwaltney et al., 1978). Apart from particle size, aerosol spread may be contingent upon nasal obstruction to divert secretions from the nares to contaminate saliva; the presumptive source of virus in sneezes and coughs (Douglas et al., 1966b). When exposed to 10 L of a small-particle aerosol, 10^1 $TCID_{50}$ of HRV-15 was associated with fever and prominent tracheobronchitis in antibody-free (<1:2) adult volunteers but not when delivered via nasal drops or a coarse aerosol (Cate et al., 1965).

7. Clinical features and disease burden associated with rhinovirus detection

HRVs have been implicated in many respiratory tract-related disease entities but illness is usually more frequent, severe and longer-lasting in children, the immunocompromized and the elderly than in healthy immunocompetent adults (Atmar, 2005; Wald et al., 1995; Dick et al., 1967). Additionally, the presence of infants or children in a household doubles the attack rate for adults (Lidwell and Sommerville, 1951).

The way in which symptoms are used to define illness and influence specimen collection are sources of variability that affect not only studies addressing the impact of HRV replication on the host but also investigations of common cold treatments (Gwaltney et al., 1996). Illness has been defined by the presence of one or more symptoms for any time period (Winther et al., 2006), for a minimum consecutive 48 h period (Spence et al., 2007), or when symptoms score above a predefined threshold (Turner et al., 1998) perhaps preceded by suitably met clinical criteria (Johnston et al., 1995). Specimens may be collected during an SRI (Spence et al., 2007; Johnston et al., 1995), 2–3 times per week for laboratory analysis (Minor et al., 1974a) or twice daily for physiological measurements of respiratory function (Johnston et al., 1995). The conclusion of an SRI, or period between SRIs, can range from seven symptom-free days (Rosenbaum et al., 1971) to ≥3 such days (Lambert et al., 2007). HRV identifications will be under-reported if clinical criteria alone are used to define viral activity (Fig. 4). Symptom score-cards and diaries maximize prospective collection of home-based data between visits by a study investigator (Johnston et al., 1995; Lambert et al., 2007) but home-based symptom recording can falter because of difficulty identifying illness or doing so in a timely manner. The shortcomings of these subjective methods can be identified and minimized by employing more frequent laboratory analysis (Winther et al., 2006; Johnston et al., 1995).

The costs of ARTIs are recognized in terms of patient and family expenditure including prescription and non-prescription medications and general practitioner's visits. Healthcare sector costs include drug and consultation subsidies, specialized hospital equipment purchases and emergency department resources while other, no less important costs including time spent away from the carer's usual activities, the reduction in carer's performance efficiency and losses in productivity are also considerable.

7.1. Upper respiratory tract illnesses

The common cold is principally a self-limiting, subjective, coryzal illness which creates a sizable annual economic burden (Lidwell and Sommerville, 1951; Jackson and Dowling, 1959; Turner, 1998). Colds are the principal outcome attributed to HRV infection but there is no obvious pattern to the symptoms reported (Lidwell and Sommerville, 1951; Bloom et al., 1963; Gern et al., 2000). More than 80% of common colds have been temporally associated with the detection of respiratory picornaviruses, of which the majority are HRVs (Andrewes, 1964; Arruda et al., 1997). However, other respiratory viruses are also associated with an URT syndrome of illness (Johnson et al., 1964) including the HCoVs (around 8% of detections), IFVs (5–15%), HRSV and the human adenoviruses (Andrewes, 1964, 1966; Pizzichini et al., 1998; Eccles, 2007). HRV strains, detected using PCR of specimens from children followed over the first year of life, increase the risk of URT illness more than the presence of HRSV (Kusel et al., 2006). Symptoms, which develop within days (Holmes et al., 1976), can include sneezing, nasal discharge (rhinorrhoea), nasal congestion/blockage, sore or irritated throat, headache, cough, a feeling of fever or 'chilliness', headache, and malaise (Winther et al., 2006; Rosenbaum et al., 1971). Hoarseness, loss of taste and smell, body aches and pains, mild burning of the eyes and a feeling of pressure in the ears or sinuses due to obstruction and/or mucosal swelling may also occur. Anorexia, loose bowels and neutrophilia have been noted among experimentally infected adult volunteers (Cate et al., 1964, 1965; Douglas et al., 1966b). Early and elevated viral shedding in adults is associated with a reduced incubation period and more severe SRI (Douglas et al., 1966b). A measurable increase in temperature is more likely to occur in children than in adults but fever can be found among either group (Dick et al., 1967; Andrewes, 1966; Miller et al., 2007). Additional clinical sensitivity may be achieved for children by sampling from episodes of atypical symptoms including uncharacteristic irritability, disturbed sleep patterns and feeding difficulties due to snuffles.

Increased antibiotic resistance among bacteria can result from unnecessarily treating large numbers of children with predominantly viral URT illnesses, expiratory wheezing, acute bronchitis (Soyka et al., 1975; Schwartz et al., 1998; Arnold et al., 2008) or mucopurulent rhinitis (a natural component of viral rhinosinusitis) (Pitkäranta et al., 1997). Antibiotics are often prescribed to assure parents and the physician that something is being done and yet these conditions derive little or no benefit from anti-bacterial interventions (Miller et al., 1960; Nyquist et al., 1998; Soyka et al., 1975; O'Brien et al., 1998). The use of antibiotics to treat these self-limiting illnesses is unwarranted since side-effects

can have a more pronounced negative, rather than positive, impact on health (Gadomski, 1993; Rosenstein et al., 1998; Todd et al., 1984).

7.2. Acute otitis media

AOM is the most frequent reason for outpatient antibiotic therapy in the United States and, until recently, was considered principally of bacterial aetiology (Heikkinen and Chonmaitree, 2003). However, URT infections involving bacteria more commonly accompany viral detection (Lehtinen et al., 2006). The isolation and PCR detection of viruses from middle ear fluids (Evans et al., 1975), failure to grow bacteria from HRV-positive fluids even in the absence of antibiotic treatment (Sung et al., 1993) and the refractory nature of some AOM cases to antibiotic therapies indicated viruses played some role in illness. Picornaviruses have been detected in 30% of nasopharyngeal swabs taken from symptomatic and asymptomatic AOM-prone infants and young children and large quantities of HRV RNA have been detected by *in situ* hybridisation of adenoid tissues from children with recurrent AOM (Pitkäranta et al., 2005; Rihkanen et al., 2004). Furthermore, HRVs have been strongly associated with AOM (Vesa et al., 2001).

7.3. Lower respiratory tract illnesses

The clinical consequences of a first infection by most respiratory viruses are more severe than those following repeat infection (Ketler et al., 1962). Acute LRT infections contribute to more morbidity and mortality than HIV infection, malaria, cancer or heart attack (Mizgerd, 2006). In particular, the importance of HRV infection in LRT morbidity during the first year of life is underappreciated (Kusel et al., 2006). Because of their small-calibre immature airways, neonates and infants are more susceptible to poor outcomes from SRIs as a result of airway swelling, excessive secretions and smooth muscle contraction during ARTI (Table 5) (Bardin et al., 1992). If HRVs naturally replicate in the LRT (Papadopoulos et al., 1999b), then a local inflammatory effect is a feasible pathogenic mechanism and evidence exists for HRV replication in non-nasal tissues including smooth muscle (Hakonarson et al., 1998) and bronchial epithelial cells (Gern et al., 2008; Jakiela et al., 2007). If not local, then the effect must be remotely triggered by URT HRV infection with concomitant systemic transmission of the immunopathologic effects (Bardin et al., 1992) or translocation of the mediators into the LRT. While HRVs have been associated with 3-fold more LRT and wheezy LRT illnesses than HRSV (Kusel et al., 2006), the risk of some LRT illnesses is similar whether an HRV, HRSV or both, are detected (Aberle et al., 2005); co-detection has been associated with more severe LRT illness than detection of HRV alone (Aberle et al., 2005). A sixth of HRV isolate-positive young children exhibit symptoms of LRT illness (mostly wheezing) and the frequency of isolation rises when underlying diseases involving the immune or cardiorespiratory systems exist (Krilov et al., 1986). In adults ≥40 years of age, the duration of symptoms and frequency of LRT illness associated with HRV isolation increase with age, peaking at nearly two-thirds of their total respiratory illness burden (Monto et al., 1987).

Table 5
Examples of LRT symptoms in populations positive for HRVs

Study category (HRV frequency, %)	Age (years)
GP (24) (Andeweg et al., 1999)	0– >64
GP (3) (Glezen et al., 1971)	Mostly 0–11
Outpatient clinic (3) (Bloom et al., 1963)	Infants and young children
Cystic fibrosis (18) (Collinson et al., 1996)	Children and adults
Virus hunting (31) (Ketler et al., 1962)	0–7
Experimental (15) (Douglas et al., 1966b)	Adult
Hospitalized with bronchiolitis (21) (Andréoletti et al., 2000)	<1
Hospitalized (Stott et al., 1969)	0–10
Hospitalized with LRT illness (Aberle et al., 2005)	0–2
Hospitalized with LRT illness (Krilov et al., 1986)	0–155

GP, general practitioner visits; ILI, influenza-like illness; LRT, lower respiratory tract, illnesses include tracheobronchitis, bronchitis, bronchiolitis, laryngotracheobronchitis (croup) and pneumonia.

Studies of children in hospital-based populations usually report more significant clinical outcomes, especially those relating to the LRT (El-Sahly et al., 2000). While conclusions should be interpreted cautiously, these data can be considered a condensed sampling of illness among community-based populations. While LRT illness has been identified in other age and patient groups (Table 5), hospital-based populations retain special importance for probing the potential of a virus to cause severe clinical outcomes, especially due to a first infection, and these cases have the strongest influence on future prioritization of therapeutic developments (Glezen and Denny, 1973).

Acute wheezing episodes (including bronchiolitis and acute asthma which share similar pathologies) are a common, epidemic and seasonal LRT manifestation of respiratory virus infection of the URT and LRT of children from all ages, but especially among males and during the first year of life (Rakes et al., 1999; Glezen et al., 1971; Henderson et al., 1979; Glezen and Denny, 1973). The mechanisms underlying the induction or exacerbation of asthma are not yet fully understood (Bardin et al., 1992; Martinez, 2007) but wheezing is blamed for excessive use of antibiotics, for being the primary cause of hospitalization among children and rarely, for death (Jartti et al., 2004b; Mallia and Johnston, 2006; Pattemore et al., 1992). Exacerbations of asthma and COPD are often preceded by a symptomatic rather than asymptomatic HRV episode (Heymann et al., 2005; Pattemore et al., 1992; Lidwell and Sommerville, 1951; Green et al., 2007; Johnston et al., 1995; Minor et al., 1974b) although in some instances, an exacerbation is the only evidence of infection

(Roldaan and Masural, 1982). Reduced peak expiratory volume in children is associated with viral detections, especially of respiratory picornaviruses (Johnston et al., 1995).

Bacterial infection was once thought to play a direct and central role in expiratory wheezing but today it is known that acute asthma exacerbations in all age groups are most often ascribed to HRV infection (El-Sahly et al., 2000; Johnston et al., 1995; Silva et al., 2007). The presence of potential bacterial pathogens cannot always be reliably correlated with LRT, or sometimes URT symptoms, because of equivalent isolation frequencies from well children (Glezen and Denny, 1973).

Significantly higher rates of HRV and more obvious LRT symptoms are detected in asthmatic children than in non-asthmatic populations (Nicholson et al., 1993; Minor et al., 1974a; Rawlinson et al., 2003; Pattemore et al., 1992). Asthma in children also appears to be a risk factor for more frequent symptomatic viral infections. However, the presence of atopy or allergy does not appear to be a common feature (Pattemore et al., 1992; Rakes et al., 1999) since only a small proportion of allergic children have asthma (Yoo et al., 2007). It is unclear if the risk of atopic asthma during infancy is increased by SRIs which affect the development of the immune system, or whether SRIs trigger asthma development in children with a genetic predisposition to more severe outcomes following HRV infection (Hershenson and Johnston, 2006; Gern and Busse, 2002; Martin et al., 2006).

HRSV infection has also been associated with causing expiratory wheezing because the virus can infect the LRT, yet reports indicate periods of epidemic wheezing unaccompanied by high rates of HRSV detection (Lemanske, 2002; Henderson et al., 1979). A similar situation has been reported for HMPV infection (Rawlinson et al., 2003). The Childhood Origins of Asthma Study (COAST) used sampling criteria that were designed to intentionally investigate the role of HRSV in illness, but the data indicated HRVs, not HRSV, were the most important predictor of subsequent wheezing in early childhood (van der Zalm et al., 2006; Lemanske et al., 2005). Although total SRIs did not differ significantly, asthmatics had more HRV infections, while their siblings had more bacterial infections. Since asthmatics are more often treated with antibiotics, bacterial detection rates may be falsely lowered in some reports (Minor et al., 1974a).

Inhaled corticosteroids are effective at suppressing airway inflammation, but less effective at preventing exacerbations initiated after HRV infection (Mallia and Johnston, 2006). New experimental human and mouse models of HRV-induced disease may provide further evidence of a causal role in LRT symptoms and also prove useful for testing therapeutic efficacy (Mallia et al., 2006; Bartlett et al., 2008).

8. Rhinoviruses and immunobiology: weapons of mass induction?

In contrast to Influenzavirus A (FLUAV) and HRSV, HRV infection involves relatively few cells and imparts little damage upon them. It is the immune response to HRV infection that is thought to drive illness (Dreschers et al., 2007; Hendley, 1998; Sung et al., 1993; Turner et al., 1998).

8.1. Cellular immunity and rhinovirus infection

Ex vivo studies of peripheral blood mononuclear cells from normal and asthmatic subjects identify a shift towards a T helper cell-2 (T_H-2) response after HRV infection of cells obtained from the asthmatic individuals. The T_H-2 response manages humoral immunity and stimulates B cells via interleukin(IL)-4 (initiating production of IgE), IL-5 (influencing eosinophils) and IL-13 (a crucial component of allergen-induced asthma). The T_H-1 response manages cellular immunity and produces IL-2 and IFN-γ. These two T cell responses act in concert with epithelial-derived chemokines (e.g. eotaxin) to promote the recruitment and activation of eosinophils and mast cells, contributing to chronic airway inflammation and the hyper-responsiveness of airways to a variety of non-specific stimuli (Gern and Busse, 2002). Although it is upregulated by HRV infection, the T_H-1 response in asthmatics is comparatively deficient (Papadopoulos et al., 2002b; McFadden, 2003; Wark et al., 2005). An increased T_H-1-like cytokine response, deduced from higher sputum mRNA IFN-γ/IL-5 values, clears HRV and more rapidly resolves symptoms (Gern et al., 2000). Induction of the type III IFNs, IFN-λ1 and IFN-λ2/3, is also impaired in bronchial epithelial cells from asthmatic but not normal subjects in whom levels are inversely associated with HRV load and symptom severity (Contoli et al., 2006). IFN-γ-induced protein 10 and RANTES are induced by HRV replication in bronchial cells; the former protein is a useful biomarker of virus-induced acute asthma associated with more severe airflow obstruction and reduced response to bronchodilators (Wark et al., 2007). Rising G-CSF and IL-8 (chemo-attractant for neutrophils) levels in the URT (protein detection in nasal wash) and LRT (mRNA in sputum) accompany rises in blood and nasal neutrophil numbers and in symptom severity in normal and atopic individuals (Gern et al., 2000; Cate et al., 1964; Levandowski et al., 1988; Turner et al., 1998). HRV binding to ICAM-1 may modify neutrophil migration and T lymphocyte-mediated cytotoxic or T_H interactions with HRV-infected cells by upregulating receptor expression (Hakonarson et al., 1998). Apart from proasthmatic changes to the responsiveness of the tissue, ICAM-I induction may also encourage eosinophil and T cell infiltration into the lower airways of asthmatic individuals and disrupt normal neutrophil trafficking potentiating the bacterial infections seen in AOM (Staunton et al., 1989; Martin et al., 2006; Sung et al., 1993).

So cells from asthmatics release more inflammatory mediators than cells from non-asthmatics and yield higher viral loads. One possible cause of the T_H-1 deficiency in asthmatics is inadequate maturation of type I and III IFN responses due to reduced exposure to infections early in life (Johnston, 2007). The ‘hygiene hypothesis’ (Strachan, 1989) posited a

pathway for the development of allergic diseases such as hay fever and asthma, conditions rising in prevalence in the post-industrial world. The suggestion is that paediatric infections play some role in preventing allergic disease so it is noteworthy that children cared for in day and group care settings have more infections than those cared for at home (Wald et al., 1988). A mechanism addressing the hygiene hypothesis is eloquently described by Gern and Busse (2002) in terms of the young, unchallenged immune system, initially set towards a T_H2-like response being dependent on infections to stimulate the development of its T_H1-like functions. One intriguing theory is that HRV infections play a central role in developing an efficacious antiviral immunity, particularly in infancy, via their ubiquitous, frequent and usually mild, self-limiting infections (Yoo et al., 2007).

8.2. *Humoral response to rhinovirus infection*

Older children and adults have greater amounts of HRV-neutralizing antibody than younger children (Mogabgab and Pelon, 1957) so the use of older subjects in many studies may have limited the observation of serious symptomatic outcomes because protective or partially cross-protective antibodies have a moderating effect. Consequently, quantifying levels of strain-specific serum antibody became routine practice prior to study commencement. Adult volunteer infection studies determined that no infections resulted in volunteers with pre-existing neutralizing antibody titres ≥1:16 and as levels grew from 0, so did levels of resistance to infection (Dick et al., 1967; Hendley et al., 1972). Nonetheless, adults could be protected by serum titres of 1:3–1:8 (Hendley et al., 1969; Dick et al., 1967) with a general trend toward decreasing numbers of SRIs with increasing age. The trend is broken by a spike in isolates from adults in the 20–39 years age group, presumably those having the children who acquire and then bring currently circulating strains into the household (Monto et al., 1987).

Secreted anti-HRV antibody, mostly IgA, appears at about the same time as serum antibody (2 weeks after inoculation of healthy adult volunteers) and is retained at peak levels for at least 8 weeks (Cooney and Kenny, 1977; Cate et al., 1966). The IgA response does not appear to modify illness or virus shedding but protects against infection and re-infection if in sufficient titre. Other studies have identified that adults, without pre-existing nasal antibody to an experimental challenge virus, may succumb to more severe SRI and shed more virus for longer (Holmes et al., 1976; Buscho et al., 1972). Antibody levels in nasal washes fall comparatively faster than serum levels (Buscho et al., 1972), especially in isolated populations, which might explain why volunteers with pre-study serum antibody (but presumably no IgA) can still become infected (Dick et al., 1967; Cate et al., 1965; Holmes et al., 1976). Importantly, there is evidence for some degree of nasal immune memory (Buscho et al., 1972).

9. Treating the wounded: the therapeutic arsenal

Symptomatic treatment with analgesics, decongestants, antihistamines, and antitussives is the most popular aim of

Table 6
Preventative and therapeutic approaches targeting rhinovirus infections

Therapeutic agent	Action	Effect	Evaluation	Ref
IFN-α	Elicit cellular antiviral effects	Decreased shedding if administered within 24hr	Toxicity	(Rotbart, 2002; Couch, 1984; Atmar, 2005; Hayden and Gwaltney, 1984; Hayden et al., 1983)
Pirodavir (R77975)	Capsid binder	Intranasal formulation useful against both antiviral groups; 3–6 doses per day	Variable efficacy, irritation and mucosal bleeding	(Hayden et al., 1992; Rotbart, 2002)
WIN 54954	Capsid binder	Broadly active in mice	Reduced efficacy in humans	(Rotbart, 2002; Patick, 2006)
Pleconaril	Capsid binder	Resolved symptoms 1–2 days earlier than placebo. Some strains are resistant.	FDA issued 'not approvable' letter because of side effects	(Rotbart, 2002)
BTA798	Capsid binder	Potent binding in animal models.	Good bioavailability and safety profile in animals. Phase I trial complete.	(Ryan et al., 2005)
Rupintrivir (formerly AG-7088)	3C protease inhibitor	Insignificant impact	Discontinued	(Patick et al., 2005; Rotbart, 2002; Wang and Chen, 2007; Binford et al., 2007)
Enviroxime	3A	Potent anti-replicative activity *in vitro*	Side-effects *in vivo*	(Couch, 1984)
Tremacamra	soluble ICAM-1 molecule	Could reduce experimental cold symptoms and the quantity of virus shed if administration occurs before or after inoculation but prior to the development of symptoms		(Turner et al., 2007)

FDA United States' Food and Drug Administration.

HRV-related intervention, although the use of aspirin and paracetamol has been associated with reduced production of neutralizing antibody, an increase in nasal signs and symptoms and in some, an increased likelihood of prolonged viral shedding (Graham et al., 1990). However data are limited on the effectiveness of over-the-counter common-cold medications for children (Smith and Feldman, 1993). The interruption of pro-inflammatory immune responses or specific signalling pathways using steroids may prove to be a more robust approach for treating HRV infections, but has not been successful for other respiratory viruses associated with expiratory wheezing (Gern and Busse, 2002). When initiated early in the illness, a combination of antiviral and anti-inflammatory components shows promise for interrupting nasal viral replication and symptoms (Gwaltney et al., 2002).

Antiviral agents (Table 6) require early application to effectively precede the pathogenic immune response to HRV infection (Gern and Busse, 2002) and often fail to reproduce their *in vitro* successes, *in vivo*. Additionally, oral delivery can complicate drug safety because this route increases the risk of systemic side effects compared to a nasal or topical route. A systemic route is beneficial if an effect is sought on virus replication sites that are otherwise inaccessible, such as those not associated with respiratory tract illness (Patick et al., 2005).

Capsid-binding compounds have attracted the most interest as an antipicornaviral drug. They reportedly act by artificially stabilizing the capsid and preventing uncoating, or by altering the canyon floor to perturb receptor interactions (Rotbart, 2002). Displacement of the pocket factor (see Fig. 1) undoubtedly plays some role in these mechanisms (Rossmann et al., 2002) nonetheless efficacy is variable against different strains. Seven of the recognized 25 HRV B strains (HRV-4, -5, -42, -84, -93, -97 and -99) are naturally resistant to pleconaril (Ledford et al., 2005) while all HRV As are sensitive. Two of the amino acid positions within VP1 known to interact with pleconaril, 152 and 191, have been implicated in this natural resistance; Phe_{152} and Leu_{191} which replace Tyr_{152} and Val_{191} (Ledford et al., 2004), respectively. Position 191 alone confers the most significant impact and different permutations of substitutions create a gradient of resistance; change in both positions confers complete resistance, demonstrated by the resistant HRV B strains (Ledford et al., 2005).

Because antivirals must be taken early and frequently to have a significant impact on illness, prophylactic use of highly sequence-specific treatments may be undesirable due to the likely development of antiviral resistance. Considering that it may be days before patients seek medical care for an ARTI (Linder, 2007), despite a belief that an impending cold is identifiable within 24 h of the first symptom (Gwaltney, 2002), it seems unlikely that any drug will be used to suitable effect. Antiviral therapeutics are currently best employed for compassionate use in populations at high risk of a poor clinical outcome.

10. New fronts in a very cold war

Unserotypeable HRV isolates had been reported prior to the 1990s (Krilov et al., 1986; Monto et al., 1987) and with the advent of PCR, the objective and rapid identification of unassigned/untypeable picornaviruses (UPVs) continued on a molecular level (Fig. 5; (Johnston et al., 1993b; Deffernez et al., 2004; Savolainen et al., 2002; Loens et al., 2006; Arden et al., 2006; Lamson et al., 2006; Jartti et al., 2004a)). We initiated a retrospective PCR-based investigation of respiratory specimens and identified a dichotomous cluster of around a dozen potential UPVs (Arden et al., 2006; McErlean et al., 2007), sharing limited identity with any subgenomic coding sequence residing on GenBank. We proposed that these belonged to HRV strains comprising a novel clade of the HRV A species, which we called HRV A2 (Arden et al., 2006). Similar subgenomic sequences were subsequently found in the United States (Lamson et al., 2006; Renwick et al., 2007) indicating a global distribution to these putative new HRVs since there are significant similarities with the first UPV sequences described. Our investigations resulted in the complete polyprotein coding sequence (Fig. 6a) of an HRV A2 strain, HRV-QPM. It proved to be the first such identification and characterization performed solely by molecular means and the first novel HRV described, although not yet isolated, in two decades. The complete coding sequences of similar, unculturable strains were subsequently reported; two 'HRV-Xs' from an adult asthma study in the United States (Kistler et al., 2007a) and three 'HRV-Cs' detected from a paediatric population studied for HBoV, by Lau et al. (2007) in Hong Kong.

The genomes of the HRV C strains are shorter and have a comparatively higher G + C content (biased towards the wobble position of certain codons) than the known HRVs. Both the P1 and P3 regions share greater amino acid identity with members of HRV A, while the $2C^{PRO}$ region is more similar to that from HRV B strains (Fig. 6b). No signs of recombination have been identified to date but the HRV-QPM sequence is so far the only divergent HRV to have been investigated in detail (McErlean et al., 2007). Across the subgenomic regions currently employed to assign species to the genus *Rhinovirus* (P1, 2C and 3CD), HRV C-like strains are genetically distinct, sharing only 53–57% average amino acid identity with the nearest species while retaining 62–98% identity with each other. Ascribing these strains to a species is strictly the purvey of the ICTV however these and other features strongly suggest that strains in the previously defined HRV A2 clade should be redefined as a novel species of HRV, which could be entitled *Human rhinovirus C* (HRV C); as previously initiated (Lau et al., 2007), further expanded upon (McErlean et al., 2008) and under independent consideration by the picornavirus study group (http://www.picornastudygroup.com/). Nonetheless, there are several traditional phenotypic traits including acid stability, neutralization pattern, receptor usage and antiviral susceptibility that cannot currently be qualified, delaying reliable taxonomic placement. We sought

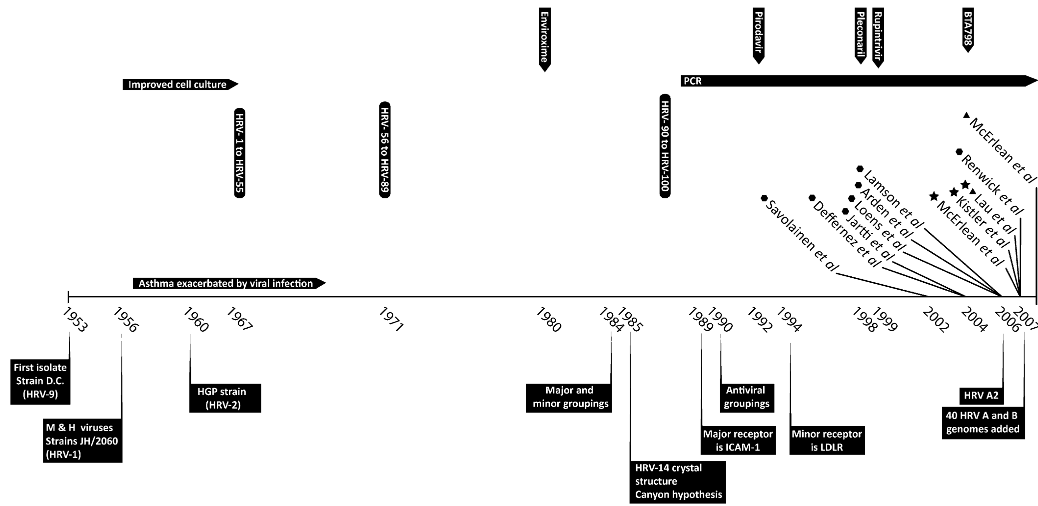


Fig. 5. A distillation of some significant events in the history of HRV research. Antiviral milestones (downward facing arrowed boxes) recognise a significant publication but may not represent first use in humans. Hexagons-reports of untypeable/unassigned picornavirus sequences derived from PCR products; stars-complete coding sequences of HRV C strains; triangles-laboratory data suggesting a new HRV species, HRV C.

to address these by creating computer-based models of HRV-QPM.

To date, HRV-QPM is the only individual HRV strain, divergent or traditional, to be a part of a deliberate PCR-based prevalence study using a specimen population screened for 17 traditional and newly identified viruses or viral groups. The largest peaks in HRV-QPM prevalence in 2003 followed the return from school break after both second (mid-year) and

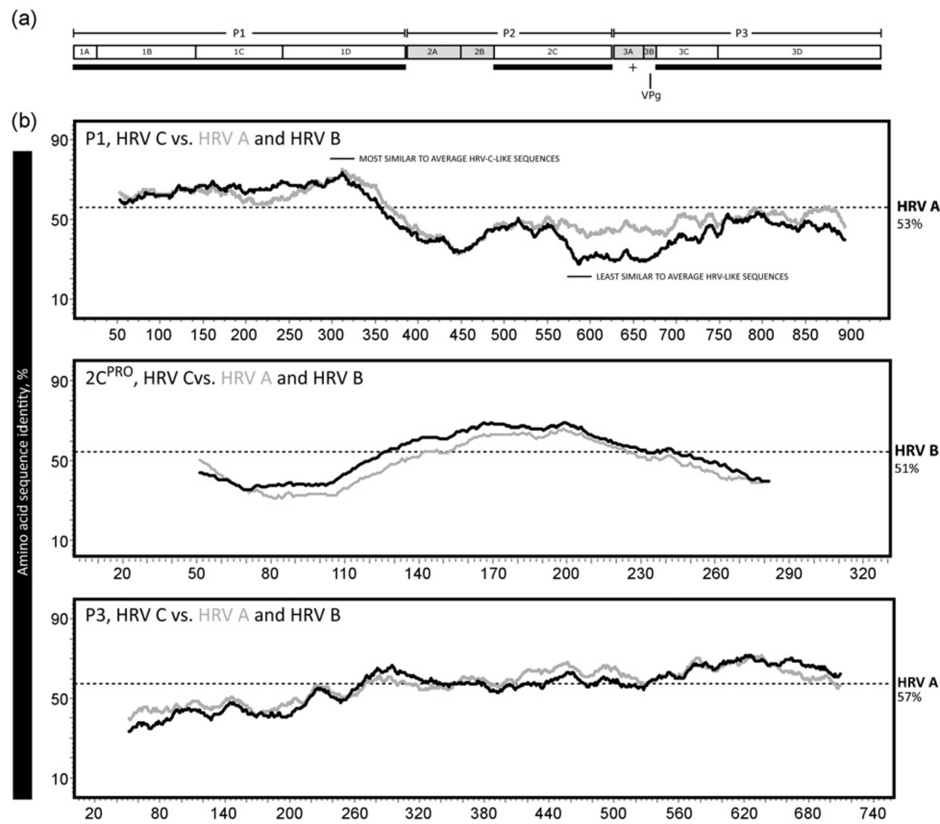


Fig. 6. Genomic features of the HRV C strains. (a) HRV genome depiction with prominent coding and polyprotein features marked, (b) plots of the average amino acid sequence identity of P1, 2C and P3 regions from all six HRV C strains compared to the same region of the other HRV species (HRV A, grey line; HRV B, black). The highest mean predicted amino acid identity is indicated by a dashed line at the specific value for the closest matching species. Constructed using SIMplot v3.2 (http://sray.med.som.jhmi.edu/SCRoftware/) using the Hamming distance model with a sliding window of 100 aa and a step of 1 aa. Adapted from (Mackay et al., in press-a).

Table 7
Clinical features among PCR-based studies describing divergent rhinovirus strains in 2007

Study group (Country)	Study age range (median)	Specimen	Most common clinical features (%)
Hospital-based[a] (Australia) (McErlean et al., 2007; McErlean et al., 2008)	1 day to 80 years (1.3 years)	NPA	Expiratory wheeze (53), persistent cough (29)
Community[b] (United States) (Lamson et al., 2006)	4 months to 98 years (25 years)	Respiratory swab	ARTI
Birth cohort (United States) (Lee et al., 2007)	0–12 months	Nasal lavage	≥5 SRI/yr
Hospitalized (HBoV study; Hong Kong) (Lau et al., 2007)	7 days to 8 years (1year)	NPA	Fever (100), expiratory wheeze (76)
With/without asthma (United States) (Kistler et al., 2007a)	Adults	Nasal lavage	URT illness
Hospitalized (Renwick et al., 2007)	Children	NPA	ARTI

ARTI, acute respiratory tract infection; LRT, lower respiratory tract; NPA, nasopharyngeal aspirate.
[a] Includes inpatients and outpatients.
[b] Visiting health care providers.

fourth (end of year) term holidays (McErlean et al., 2007). Variants were identified in infants enduring mild to severe SRIs that commonly manifested as acute expiratory wheezing (only HRV-QPM detected) or persistent cough (commonly HRV-QPM accompanied by another virus) usually requiring hospitalization and often receiving supplemental oxygen. HRV-QPM was the only virus found in 65% of its detections (McErlean et al., 2007, 2008). Investigations to date have yielded HRV-QPM variants in Europe, the United States (Lee et al., 2007) and elsewhere in Australia during 2003, indicating their global and probably endemic circulation.

It is tempting to propose that the HRV Cs may yield a source of strains more frequently associated with expiratory wheezing (Lau et al., 2007; McErlean et al., 2007) and pneumonia (undefined) (Renwick et al., 2007) but too few studies have been conducted for there to be any significant differences in clinical outcome (Table 7) and little is known about the impact of individual HRV A and B strains.

Currently, no cultivation of an HRV C strain has been reported despite inoculation of many ‘traditional’ cell lines. Our *in silico* analyses predicted that the likelihood of HRV-QPM employing a VLDL-R receptor was slim and that an ICAM-like molecule may be a more probable candidate (McErlean et al., 2008). The new strains contain deletions largely in the BC, DE and HI loops of VP1 (Fig. 2) and include Thr_{191} in VP1 which could convey natural resistance to pleconaril (Lau et al., 2007). The impact of the HRV C strains on the efficacy of current antiviral designs may be significant.

No evidence exists to suggest that the HRV C strains are emerging or ‘new’ viruses nor that their discovery requires novel molecular techniques; all that proved necessary was the vigilant characterization of UPVs. It appears HRV C strains are newly identified viruses (Mackay et al., in press-b) that have been contributing to SRIs, in the absence of detection, for at least a decade.

Intriguingly, in our hands, the Gama assay (Gama et al., 1988) continues to detect all the HRVs, even the newly identified and highly divergent strains. This is impressive considering that up until 2007, only eight complete genomes resided in the public domain, mostly appearing between 1984 and 1988. In 2007 the number of genome sequences increased to nearly 50 (Kistler et al., 2007b; Tapparel et al., 2007; McErlean et al., 2007; Lau et al., 2007; Kistler et al., 2007a). The sequences of all traditional HRV strains should be available soon (Rathe, 2008). Our interpretations derived from the literature and our ongoing studies suggest there could be as many more HRV strains circulating, ascribed to new and existing species, as there are currently known HRV strains. Furthermore, the divergent viruses may have novel or fastidious requirements for cultivation, utilize different molecules as receptors, circulate during a specific season, be unexpectedly resistant to some antivirals and perhaps be associated with specific clinical outcomes.

11. Summary

Interest in the field of rhinovirology is on the rise, and the attention is long overdue. Early studies of adults and older children perhaps defined the mild clinical impact still associated with infection by the ‘common cold’ viruses and possibly detracted from further characterization of the populous viral super-group. The emergence of HIV also undermined HRV characterization in the 1980s; the common cold could hardly compete with AIDS for research dollars (Tyrrell and Fielder, 2002). However, the emergent SARS-CoV, may have returned respiratory virus hunting to the research radar. Once working in the field, it is impossible to ignore the dominance of HRV infections in many aspects of respiratory tract illnesses. Thankfully, some researchers held their ground when HRV research was less popular, continuously producing important immunobiological and virological data, investigating a role for the HRVs in our immune development and identifying their greater impact among hosts with certain immune deficiencies. Previous studies were weakened by unreliable cultivation techniques, laborious and possibly cross-reactive serological methods and the confounding and seemingly random contribution of UPVs to HRV epidemiology. These problems confused the definition of HRV infection frequency and the extent of their clinical impact on early childhood illness. We now know that HRVs play a central

role in expiratory wheezing, contribute to the development of antibiotic resistance and are associated with hospitalization of children negative for other viruses. Such findings are driven by an improved diagnostic capacity and aided by the increasing popularity of virus hunting. Clearly, for clinical infectious disease studies to have significant power, all pathogens must first be identified and then comprehensively characterized. It is already apparent that modern tools and greater attention to the HRVs has meant that earlier associations between respiratory illness and infection by single, "traditional" viruses do not retain their significance today. Limited data comprehensively address HRV strain-specific circulation patterns, antiviral resistance, sequence variability or the possibility of robust associations between HRV strains or species and specific clinical outcomes. We also do not know the extent of their involvement in fine-tuning, or perhaps developing, aspects of our immunological defences and yet the HRVs seem to play some role (Johnston and Openshaw, 2001). Today, renewed interest in HRVs is fuelled by the molecular characterization of traditional HRV genomes and the discovery of divergent strains. Now is the time to address the unanswered questions which litter a particularly neglected battlefield in one of the many wars between viruses and their human hosts.

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