Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
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

Received 28 March 2008; accepted 4 April 2008

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.

© 2008 Elsevier B.V. All rights reserved.

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

Contents

1. Introduction ................................................................. 298
2. The human rhinovirus .......................................................... 299
   2.1. Classification schemes: strains, types groups and species ......................................................... 299
   2.2. The rhinovirion .......................................................... 300
3. HRV strains: more than meets the eye? .................................................. 301
   3.1. Host immunity: raising the iron curtain .......................................................... 301
   3.2. Sequence conservation: time may not weary them ............................................................... 301
   3.3. Clinical outcome: too early to dismiss strain-specific clinical features? ............................. 301
4. Forty years of rhinovirus detection .......................................................... 302
   4.1. Cell culture methods .......................................................... 302
   4.2. Polymerase chain reaction: weapon of choice for rhinovirus detection ............................. 303
5. Rhinovirus circulation patterns .......................................................... 304
   5.1. Rhinovirus infection: occupation or skirmish? .......................................................... 304
6. Rhinovirus transmission .......................................................... 306

∗ 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.

1386-6532/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jcv.2008.04.002
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 (Keltler 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 HRV strains 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 naive 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 aetiologic 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...
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 ‘polypeptide’ (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 epitopes. 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 Colonna, 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 Colonna, 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       | 34H | 1M |
| 2       | 36  | 4  |
| 3       | 38H | 5  |
| 4       | 39  | 6H |
| 5       | 40H | 9H |
| 6       | 41  | 10H|
| 7       | 43  | 11H|
| 8       | 44  | 12H|
| 9       | 45  | 13H|
| 10      | 46  | 15H|
| 11      | 47  | 16H|
| 12      | 49H | 18H|
| 13      | 50  | 19H|
| 14      | 51H | 20H|
| 15      | 53  | 21H|
| 16      | 54  | 22H|
| 17      | 55  | 23H|
| 18      | 56  | 24H|
| 19      | 57  | 25H|
| 20      | 58  | 26H|
| 21      | 59  | 28H|
| 22      | 60  | 29H|
| 23      | 61  | 30H|
| 24      | 62  | 31H|
| 25      | 63  | 33H|

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; Kettler 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 (Munnaughton, 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; Hanre, 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 Colonna, 1984; Colonna 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. 

Northern face (Fig. 1) employing a different destabilization 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 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
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 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 (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, 1961). At the same time, propagation of the HGP 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.
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) (Blom 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, 3, 7, 64, SD-7407 | 1971 |
| Adult (Office workers) (Gwaltney et al., 1966) | ARTI | 3, 27, 64, SD-7407 | 1966 |
| Adult (Lung transplant) (Kaiser et al., 2006) | LRT illness | 1B, 3, 55 | 2006 |
| Adult (Military) (Bloom et al., 1963) | URT illness | 2, 1B, 1D | 1963 |
| Adult (Isolation) (Holmes et al., 1976) | LRT illness | 1B, 19, 23, 74 | 1976 |
| Children (isolation) (Stott et al., 1969) | LRT illness | 1B, 10, 16, 18, 19, 32, 33, 38, 40, 49, 51, 65, 78 | 1969 |
| Children (Krilov et al., 1986) | LRT illness | 1B, 44, 53, 56, 63, 88 | 1986 |
| Adult (LTCF) (Louie et al., 2005) | LRT illness | 2 | 2005 |
| Adult (Military) (Bloom et al., 1963) | URT illness | 1A, 1B, 2 | 1963 |
| Adult (Surgery) (Craighead et al., 1969) | LRT illness | 1B, 19, 23, 74 | 1969 |
| Adult (Surgery) (McGee et al., 1967) | LRT illness | 1B, 19, 23, 74 | 1969 |
| Adult (Office workers) (Gwaltney et al., 1966) | URT illness | 2 | 1969 |
| Children Adults (Reilly et al., 1962) | ARTI-Ab NEG for common viruses | 10, 12, 13, 18, 19, 23–25, 27, 28 | 1962 |
| 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 | 1976 |
| Children (Krilov et al., 1986) | LRT illness | 1B, 10, 16, 18, 19, 32, 33, 38, 40, 49, 51, 65, 78 | 1986 |
| Children Adults (Reilly et al., 1962) | ARTI-Ab NEG for common viruses | 10, 12, 13, 18, 19, 23–25, 27, 28 | 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 pediatric 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; Johnstone 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; Defernez 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; Hyytiä et al., 1989; Fremuth 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\textsubscript{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 influenza virus (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; Shamukh 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 A2 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 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    | Culture          |
| Infants (any virus) (Lemanske et al., 2006) | 7.7 per 12 months     | PCR              |
| Children (any microorganism) (Collinson et al., 1996) | <6 years, 3.4 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          | PCR              |
| Day care (Wald et al., 1988)        | 7.1 per year          | PCR              |

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 picornavirus RT-PCR

![Fig. 4. The importance of sampling time and strain typing when investigating HRV shedding.](image-url)

(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.
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 $1.6 \times 10^5$ TCID$_{50}$/mL (Douglas et al., 1966b). Experimental transmission was also reportedly inefficient (D’Alessio et al., 1984). In contrast, ‘naturally’ close-quarted 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., 1966b; Winther et al., 1986). 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 $1.6 \times 10^5$ TCID$_{50}$/mL (Douglas et al., 1966b). Experimental transmission was also reportedly inefficient (D’Alessio et al., 1984). In contrast, ‘naturally’ close-quarted 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., 1966b; Winther et al., 1986). 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 $1.6 \times 10^5$ TCID$_{50}$/mL (Douglas et al., 1966b). Experimental transmission was also reportedly inefficient (D’Alessio et al., 1984). In contrast, ‘naturally’ close-quarted 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., 1966b; Winther et al., 1986). Nasopharyngeal washes yield better viral isolation
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 contami-
nate saliva; the presumptive source of virus in sneezes and coughs (Douglas et al., 1966b). When exposed to 10 L of a small-particle aerosol, 10^3 TCID50 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 immunocompro-
mized 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 pre-
defined threshold (Turner et al., 1998) perhaps preceded by suitably met clinical criteria (Johnston et al., 1995). Speci-
mens 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 physiologi-
cal 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 identi-
fications 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 record-
ing 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 impor-
tant 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 bur-
den (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 respira-
atory 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 ade-
noviruses (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 (rhinor-
hoea), 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; Andrews, 1966; Miller et al., 2007). Additional clinical sensitivity may be achieved for children by sampling from episodes of atypical symptoms includ-
ing 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 natu-
ral 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 inter-
ventions (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 (Pitkärinta 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; Jakiel 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).

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. Although it is upregulated by HRV infection, the TH-1 response in asthmatics is comparatively deficient (Papadopoulos et al., 2002b; McFadden, 2003; Wark et al., 2005). An increased TH-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-α 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 (pro-inflammatory mediator) 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). 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 (TH-2) response after HRV infection of cells obtained from the asthmatic individuals. The TH-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 TH-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 TH-1 response in asthmatics is comparatively deficient (Papadopoulos et al., 2002b; McFadden, 2003; Wark et al., 2005). An increased TH-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-α 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 cytotoxicity or TH cell interactions with HRV-infected cells by upregulating receptor expression (Hakonarson et al., 1998). Apart from proinflammatory changes to the responsiveness of the tissue, ICAM-1 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 TH-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 TH2-like response being dependent on infections to stimulate the development of its TH1-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 \( \geq 1:16 \) 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).

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 Pleconaril | Capsid binder | Broadly active in mice Resolved symptoms 1–2 days earlier than placebo. Some strains are resistant. | Reduced efficacy in humans FDA issued ‘not approvable’ letter because of side effects | (Rotbart, 2002; Patick, 2006) |
| BTA798 Pleconaril | 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; Phe152 and Leu191 which replace Tyr152 and Val191 (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; Deffenez 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, unculturatable 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 2CPRo 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 purview 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).
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 Thr191 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, continuing to develop 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 both early childhood illness and asthma, making them an important area of research.
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.

Acknowledgments

This review was possible because of NHMRC support, grant (455905). Thanks go to Sebastian Johnston, Philip Pattemore, Robert Atmar, Nick Knowles, Laurent Kaiser, Caroline Tapparel, Wai-Ming Lee, James Gern, Jane Ryan, Thomas Briese and David Wang for their generosity in providing answers to many questions. Special thanks to Cassandra E. Faux, Peter K. McErlean and Katherine E. Arden for data and discussion.

References

PCR. Real-Time PCR in microbiology: from diagnosis to characterization. Norfolk: Caister Academic Press; 2007.
Aberle JH, Aberle SW, Pracher E, Hutter H-P, Kundi M, Popw-Kraupp T. Impact on clinical course of disease and interferon-g response. Pediatr Infect Dis J 2005;24:605–10.
Abraham G, Colombo RJ. Many rhinovirus serotypes share the same cellular receptor. J Virol 1984;51:340–5.
Al-Sunaidi M, Williams CH, Hughes PJ, Schnurr DP, Stanway G. Analysis of a new human parechovirus allows the definition of parechovirus types and the identification of RNA structural domains. J Virol 2007;81:1013–21.
Andeweg AC, Bestebroer TM, Huybrechs M, Kimman TG, de Jong JC. Improved detection of rhinoviruses in clinical samples by using a newly developed nested reverse transcription-PCR assay. J Clin Microbiol 1999;37:524–30.
Andréolletti L, Lesay M, Deschilde A, Lambert V, Dewilde A, Wattré P. Differential detection of rhinoviruses and enteroviruses RNA sequences associated with classical immunofluorescence assay detection of respiratory virus antigens in nasopharyngeal swabs from infants with bronchiolitis. J Med Virol 2000;61(3):341–6.
Andrews CH. The complex epidemiology of respiratory virus infections. Science 1964;146:1274–7.
Andrews CH. Rhinoviruses and common colds. Ann Rev Med 1966;17:361–70.
Andrews CH, Chaproniere DM, Gompels AEH, Pereira HG, Roden AT. Propagation of common-cold virus in tissue cultures. Lancet 1953;265:546–7.
Andries K, Dewindt B, Snoeks J, Wouters L, Moeheels H, Lewi PJ, et al. Two groups of rhinoviruses revealed by a panel of antiviral compounds present sequence divergence and differential pathogenicity. J Virol 1990;64:1117–23.
Arden KE, McErlean P, Nissen MD, Sloots TP, Mackay IM. Frequent detection of human rhinoviruses, paramyxoviruses, coronaviruses, and bocavirus during acute respiratory tract infections. J Med Virol 2006;78:1232–40.
Arnold JC, Singh KK, Spector SA, Sawyer MH. Undiagnosed respiratory viruses in children. Pediatrics 2008;121:e631–7.
Arruda E, Hayden FG. Detection of human rhinovirus RNA in nasal washings by PCR. Mol Cell Probes 1993;7:373–9.
Arruda E, Pitkäranta A, Witek TJ, Doyle CA, Hayden FG. Frequency and natural history of rhinovirus infections in adults during autumn. J Clin Microbiol 1997;35:2864–8.
Atmar RL. Uncommonly manifested infections of rhinovirus, agent of the common cold. Clin Infect Dis 2005;41:266–7.
Baer G, Schaad UB, Heininger U. Clinical findings and unusual epidemiologic characteristics of human metapneumovirus infections in children in the region of Basel, Switzerland. Eur J Pediatr 2007;167:63–9.
Banham TM. A collaborative study of the aetiology of acute respiratory infections in Britain 1961–4. A report of the medical research council working party on acute respiratory virus infections. Br Med J 1965;2:319–26.
Bardin PG, Johnston SL, Pattemore PK. Viruses as precipitants of asthma symptoms II. Physiology and mechanisms. Clin Exp Allergy 1992;22:809–22.
Barrett B, Brown R, Voland R, Maberry R, Turner RB. Relations among questionnaire and laboratory measures of rhinovirus infection. Eur Respir J 2006;28:358–63.
Bartlett NW, Walton RP, Edwards MR, Aniscenko J, Caramori G, Zhu J, et al. Mouse models of rhinovirus-induced disease and exacerbation of allergic airway inflammation. Nat Med 2008;14:199–204.
Behbehani AM, Lee LH. Growth, plaque production and cationic stabilization of rhinovirus type 1 (Echovirus 29). J Bacteriol 1964;88:1608–11.
Bertino JS. Cost burden of viral respiratory infections: Issues for formulary decision makers. Am J Med 2002;112:42S–9S.
Billand G, Peny S, Legay V, Lina B, Valette M. Detection of rhinovirus and enterovirus in upper respiratory tract samples using a multiplex nested PCR. J Virol Methods 2003;108:223–5.
Binford S, Weady PT, Maldonado F, Brothers MA, Matthews DA, Patt-ick AK. In vitro resistance studies of Rupintrivir, a novel inhibitor of human rhinovirus 3C protease. Antimicrob Agents Chemother 2007;51:4366–73.
Blomqvist S, Skyttä A, Rovainen M, Hovi T. Virological and serological analysis of rhinovirus infections during the first two years of life in a cohort of children. J Med Virol 2002;66:263–8.
Blomqvist S, Skyttä A, Rovainen M, Hovi T. Rapid detection of human rhinoviruses in nasopharyngeal aspirates by a microwell reverse transcription-PCR-hybridization assay. J Clin Microbiol 1999;37:2813–6.
Bloom HH, Forsyth BR, Johnson KM, Chanock RM. Relationship of rhinovirus infection to mild upper respiratory disease. J Am Med Assoc 1963;186:38–45.
Briese T, Palacios G, Kokoris M, Jabado O, Liu Z, Renwick N, et al. Diagnostic system for rapid and sensitive differential detection of pathogens. Emerg Infect Dis 2005;11:310–3.
Brunstein JD, Cline CL, McKinney S, Thomas E. Evidence from multiplex molecular assays for complex multipathogen interactions in acute respiratory infections. J Clin Microbiol 2008;46:97–102.
Bryce I, Boschi-Pinto C, Shibuya K, Black RE. WHO estimates of the causes of death in children. Lancet 2005;365:1147–52.

Buscho RF, Perkins JC, Knopf HLS, Kapikian AZ, Chanock RM. Further characterization of the local respiratory tract antibody response induced by intranasal instillation of inactivated rhinovirus 13 vaccine. J Immunol 1972;108:169–77.

Cate TR, Dougher JR, Fleet WF, Griffith WR, Gerone PJ, Knight V. Production of tracheobronchitis in volunteers with rhinovirus in a small-particle aerosol. Am J Epidemiol 1965;81:95–105.

Cate TR, Dougher RB, Johnson KM. Studies with rhinoviruses in volunteers: production of illness, effect of naturally acquired antibody, and demonstration of a protective effect not associated with serum antibody. J Clin Invest 1964;43:56–67.

Cate TR, Rossen RD, Douglas Jr RG, Butler WT, Couch RB. The role of nasal secretion and serum antibody in the rhinovirus common cold. Am J Epidemiol 1966;84:352–63.

Coiras MT, Aguilar JC, García ML, Casas I, Pérez-Breña MP. Simultaneous detection of fourteen respiratory viruses in clinical specimens by two multiplex reverse transcription nested-PCR assays. J Med Virol 2004;72:484–95.

Collinson J, Nicholson KG, Cancio E, Ashman J, Ireland DC, Hammersley V, et al. Effects of upper respiratory tract infections in patients with cystic fibrosis. Thorax 1996;51:1115–22.

Colombo RJ, Callahan PL, Long WJ. Isolation of a monoclonal antibody that blocks attachment of the major group of human rhinoviruses. J Virol 1986;57:7–12.

Conant RM, Hamparian VV. Identification of rhinovirus strain D.C. Nature 1968b;217:1264.

Conant RM, Hamparian VV. Rhinoviruses: Basis for a numbering system. II. Serologic characterization of prototype strains. J Immunol 1968b;100:107–13.

Contoli M, Message SD, Laza-Stanca V, Edwards MR, Wark PAB, Bartlett NW, et al. Role of deficient type III interferon-1 production in asthma exacerbations. Nat Med 2006;12:1023–6.

Cooney MK, Hall CB, Fox JP. The Seattle virus Watch. III. Evaluation of isolation methods and summary of infections detected by virus isolations. Am J Epidemiol 1972;96:286–305.

Cooney MK, Kenny GE. Demonstration of dual rhinovirus infection in humans by isolation of different serotypes in human heteroploid (HeLa) and human diploid fibroblast cell cultures. J Clin Microbiol 1975;5:702–7.

Couch RB. The common cold: control? J Infect Dis 1984;150:189–94.

Couch RB, Douglas Jr RG, Lindgren KM, Gerone PJ, Knight V. Airborne transmission of respiratory infection with Coxackievirus A type 21. Am J Epidemiol 1970;91:78–86.

Craighead JE, Meier M, Cooley MH. Pulmonary infection due to rhinovirus type 13. N Engl J Med 1969;281:1403–4.

D’ Alessio DJ, Meschievitz CK, Peterson JA, Dick CR, Dick EC. Short-duration exposure and the transmission of rhinoviral colds. J Infect Dis 1984;150:189–94.

Deffernez C, Wunderli W, Thomas Y, Yerly S, Perrin L, Kaiser L. Amplicon sequencing and improved detection of human rhinovirus in respiratory samples. J Clin Microbiol 2004;42:3212–8.

Dick EC, Blumer CR, Evans AS. Epidemiology of infections with rhinovirus types 43 and 55 in a group of university of Wisconsin student families. Am J Epidemiol 1967;86:386–400.

Douglas JR RG, Cate TR, Couch RB. Growth and cytopathic effect of H type rhinoviruses in monkey kidney tissue culture. Proc Soc Exp Biol Med 1966a;123:234–41.

Douglas JR RG, Cate TR, Gerone PJ, Couch RB. Quantitative rhinovirus shedding patterns in volunteers. Am Rev Respir Dis 1966b;94:159–67.

Dreschers S, Dumitru CA, Adams C, Gulbins E. The cold case: are rhinoviruses perfectly adapted pathogens? Cell and Mol Life Sci 2007;64:181–91.

Eccles R. Mechanisms of symptoms of the common cold and influenza. Br J Hosp Med 2007;68:578–82.

El-Sabry HM, Attmar RL, Glezen WP, Greenberg SB. Spectrum of clinical illness in hospitalized patients with “Common cold” virus infections. Clin Infect Dis 2000;31:96–100.

Evans JR FO, Sydor JB, Moore WEC, Moore GR, Manwaring JL, Brill AH, et al. Sinusitis of the maxillary antrum. N Engl J Med 1975;293:735–9.

Fiala M, Kenny GE. Enhancement of rhinovirus plaque formation in human heteroploid cell cultures by magnesium and calcium. J Bacteriol 1966;92:1715–7.

Fox JP, Cooney MK, Hall CE. The Seattle virus watch. V. Epidemiologic observation of rhinovirus infections, 1965–1969 in families with young children. Am J Epidemiol 1975;101:122–43.

Freymuth F, Vabret A, Galateau-Salle F, Ferey J, Eugene G, Petitjean J, et al. Detection of respiratory syncytial virus, parainfluenza virus 3, adenovirus and rhinovirus sequences in respiratory tract of infants by polymerase chain reaction and hybridization. Clin Diagn Virol 2000;8:31–40.

Gadomski AM. Potential interventions for preventing pneumonia among young children: lack of effect of antibiotic treatment for upper respiratory infections. Pediatr Infect Dis J 1993;12:115–20.

Gama RE, Hughes PJ, Bruce CB, Stanaway G. Polymerase chain reaction amplification of rhinovirus nucleic acids from clinical material. Nucleic Acids Res 1988;16;9346.

Gern JE, Busse WW. Relationship of viral infections to wheezing illnesses and asthma. Nat Rev Immunol 2002;2:132–8.

Gern JE, Galagan DM, Jarjour NN, Dick EC, Busse WW. Detection of rhinovirus RNA in lower airway cells during experimentally induced infection. Am J Respir Crit Care Med 2008;177:1159–61.

Gern JE, Vrits R, Grindle KA, Swenson C, Busse WW. Relationship of upper and lower airway cytokines to outcome of experimental rhinovirus infection. Am J Respir Crit Care Med 2000;162:2226–31.

Glezen WP, Denny FW. Epidemiology of acute lower respiratory disease in children. N Engl J Med 1973;288:498–505.

Glezen WP, Loda FA, Clyde WA, Senior RJ, Sheaffer CI, Conley WG, et al. Epidemiologic patterns of acute lower respiratory disease of children in a pediatric group practice. J Pediatr 1971;78:397–406.

Graham NM, Burrell CJ, Douglas RM, Deabble P, Davies L. Adverse effects of aspirin, acetaminophen, and ibuprofen on immune function, viral shedding, and clinical status in rhinovirus-infected volunteers. J Infect Dis 1990;162:1277–82.

Green RM, Custovic A, Sanderson G, Hunter J, Johnston SL, Woodcock A. Synergism between allergens and viruses and risk of hospital admission with asthma: case-control study. Br Med J 2007;334:1–5.

Greve JM, Davis G, Meyer AM, Forte CP, Yost SC, Marlor CW, et al. The major human rhinovirus receptor is ICAM-1. Cell 1989;56:839–47.

Gwaltney Jr JM. Micro-neutralization test for identification of rhinovirus serotypes. Proc Soc Exp Biol Med 1966;122:1137–41.

Gwaltney Jr JM. Viral respiratory infection therapy: historical perspectives and current trials. Am J Med 2002;112:335–41S.

Gwaltney Jr JM, Buier RM, Rogers JL. The influence of signal variation, bias, noise and effect size on statistical significance in treatment studies of the common cold. Antiviral Res 1996;29:287–95.

Gwaltney Jr JM, Hendley JO. Rhinovirus transmission one if by air, two if by hand. Am J Epidemiol 1978;107:357–61.

Gwaltney Jr JM, Hendley JO, Simon G, Jordan Jr WS. Rhinovirus infections in an industrial population I. The occurrence of illness. N Engl J Med 1966;275:1261–8.

Gwaltney Jr JM, Jordan Jr WS. Rhinoviruses and respiratory disease. Bacteriol Rev 1964;28:409–22.

Gwaltney Jr JM, Winther B, Patrie JT, Hendley JO. Combined antiviral-antimediator treatment for the common cold. J Infect Dis 2002;186:147–54.

Gwaltney Jr JM, Moskalski PB, Hendley JO. Hand-to-hand transmission of rhinovirus colds. Ann Intern Med 1978;88:463–7.

Hakonarson H, Maskeri N, Carter C, Hodinka RL, Campbell D. Gadomski VV. Relationship of upper and lower airway cytokines to outcome of experimental rhinovirus infection. Am J Respir Crit Care Med 2000;162:2226–31.

Glezen WP, Denny FW. Epidemiology of acute lower respiratory disease in children. N Engl J Med 1973;288:498–505.

Gwaltney Jr JM, Hendley JO. Rhinovirus transmission one if by air, two if by hand. Am J Epidemiol 1978;107:357–61.

Gwaltney Jr JM, Hendley JO, Simon G, Jordan Jr WS. Rhinovirus infections in an industrial population I. The occurrence of illness. N Engl J Med 1966;275:1261–8.

Gwaltney Jr JM, Jordan Jr WS. Rhinoviruses and respiratory disease. Bacteriol Rev 1964;28:409–22.

Gwaltney Jr JM, Winther B, Patrie JT, Hendley JO. Combined antiviral-antimediator treatment for the common cold. J Infect Dis 2002;186:147–54.

Gwaltney Jr JM, Moskalski PB, Hendley JO. Hand-to-hand transmission of rhinovirus colds. Ann Intern Med 1978;88:463–7.
Hurlin AF, Ghimire AK, Thompson MA, Black JF, Brand CA, Lowe AJ, Hungnes O, Jonassen TØ, Jonassen CM, Grinde B. How sequence information helps us understand the evolution and dissemination of viruses. J Clin Microbiol 1995;33:648–53.

Hamparian VV, Colombo RJ, Cooney MK, Dick EC, Gwaltney Jr JM, Hughes JH, et al. A collaborative report: Rhinoviruses—extension of the numbering system from 89 to 100. Virology 1987;159:191–2.

Hamparian VV, Kelter A, Hilleman MR. Recovery of new viruses (Coryza Virus) from cases of common cold in human adults. Proc Soc Exp Biol Med 1961;108:444–53.

Hamre D. Rhinoviruses. Monogr Virol 1967;1:1–85.

Hamre D, Connolly AP, Procknow JJ. Virologic studies of acute respiratory disease in young adults. IV. Virus isolation during four years of surveillance. Am J Epidemiol 1966;83:238–49.

Hayden FG, Andries K, Janssen PAJ. Importance of respiratory viruses in acute otitis media. Clin Microbiol Rev 2003;16:230–41.

Hayden FG, Gwaltney Jr JM. Intranasal interferon-a treatment of experimental rhinoviral colds. J Infect Dis 1984;150:174–80.

Hayden FG, Herrington DT, Coats TL, Kim K, Cooper EC, Villano SA, et al. Efficacy and safety of oral poconaril for treatment of colds due to picornaviruses in adults: results of 2 double-blind, randomized, placebo-controlled trials. Clin Infect Dis 2003;36:1523–32.

Hayden FG, Mills SE, Johns ME. Human tolerance and histopathologic effects of long-term administration of intranasal interferon-a2. J Infect Dis 1983;148:914–21.

Heikkinen T, Chonmaitree T. Importance of respiratory viruses in acute otitis media. Clin Microbiol Rev 1993;6:727–32.

Henderson FW, Clyde WA, Collier AM, Denny FW, Senior RJ, Sheaffer CI, et al. The etiologic and epidemiologic spectrum of bronchiolitis in pediatric practice. J Pediatr 1979;95:183–90.

Hendley JO. The host response, not the virus, causes the symptoms of the common cold. Clin Infect Dis 1998;26:847–8.

Hendley JO, Edmondson Jr WP, Gwaltney Jr JM. Relation between naturally acquired immunity and infectivity of two rhinoviruses in volunteers. J Infect Dis 1972;125:243–8.

Hendley JO, Gwaltney Jr JM. Mechanisms of transmission of rhinovirus infections. Epidemiol Rev 1988;10:242–58.

Hendley JO, Gwaltney Jr JM, Jordan Jr WS. Rhinovirus infections in an industrial population. IV. Infections within families of employees during two fall peaks of respiratory illness. Am J Epidemiol 1969:89;184–96.

Hendley JO, Wenzel RP, Gwaltney Jr JM. Transmission of rhinovirus colds by self-inoculation. N Engl J Med 1973;288:1361–4.

Hershenson MB, Johnston SL. Rhinovirus infections more than a common cold. Am J Respir Crit Care Med 2001;164:1284–5.

Heymann PW, Platts-Mills TAE, Johnston SL. Role of viral infections, atopy and antiviral immunity in the etiology of wheezing exacerbations among children and young adults. Pediatr Infect Dis J 2005;24:S217–22.

Hofer F, Gruenberger M, Kowalski H, Machat H, Huettinger M, Kuechler H, et al. Prevalence of viral infections, atopy and rapid identification of human picornaviruses. J Clin Virol 2004a;72:695–9.

Hiramatsu M, Ueda M, Nakamura H, Rikihisa Y. Reduction of human picornavirus RNA in the nose of acute respiratory illness in children. J Med Virol 2004b;72:316–21.

Hirschhorn MB, Johnston SL. Rhinovirus infections More than a common cold. J Infect Dis 1983;148:914–21.

Hiscoh K, Miura R, Shimada Y, Hayashi A, Nakajima H, Yamazaki S, et al. Human rhinovirus 87 Identified as human enterovirus 68 by VP4-based molecular diagnosis. Intervirology 2002;45:136–41.

Jackson GG, Dowling HF. Transmission of the common cold to volunteers under controlled conditions. IV. Specific immunity to the common cold. J Clin Invest 1959;38:762–9.

Jenkins J, Bouscambert-Duchamp M, Moret H, Carquin J, Brodard V, Lina B, et al. Association of respiratory picornviruses with acute bronchiolitis in French infants. J Clin Virol 2006;35:463–6.

Jia J, Brockman-Schneider R, Aminova S, Lee W-M, Gern JE. Basal cells of differentiated bronchial epithelium are more susceptible to rhinovirus infection. Am J Respir Cell Mol Biol 2008;38(5):517–23.

Johansen MA, Schaffer C, Gwaltney Jr JM. The September community study of role of viral infections in exacerbations of asthma among adults. Br Med J 2001;322:376–7.

Johnston SL. Innate immunity in the pathogenesis of virus-induced asthma exacerbations. Proc Am Thorac Soc 2007;4:267–70.

Johnston SL, Bardin PG, Patmore PK. Viruses as precipitants of asthma symptoms III. Rhinoviruses: molecular biology and prospects for future intervention. Clin Exp Allergy 1993a;23:237–46.

Johnson HE, Altman R, Hamre D, Ward T. Viral infections and the common cold. Chest 1964;45:46–53.

Johnson NW, Johnston SL, Norman GR, Dai J, Sears MR. The September epidemic of asthma hospitalization: school children as disease vectors. J Allergy Clin Immunol 2006;117:557–62.

Johnson SL. Persistence of human rhinovirus RNA in the nose after acute respiratory illness in children. J Med Virol 2007a;81:1225–9.

Johnson SL, Sanderson G, Patmore PK, Smith S, Lampe F, Josephs L, et al. Community study of role of viral infections in exacerbations of asthma in 9–11 year old children. Br Med J 1995;310:1225–9.

Johnson SL, Sanderson G, Patmore PK, Smith S, Bardin PG, Bruce CB, et al. Use of polymerase chain reaction for diagnosis of picornavirus infection in subjects with and without respiratory symptoms. J Clin Microbiol 1998;36:111–7.

Kaiser L, Aubert J-D, Pache J-C, Deffernez C, Rochat T, Garbino J, et al. Chronic rhinoviral infection in lung transplant recipients. Am J Respir Crit Care Med 2006;174:1392–9.

Kämmerer U, Kunkel K, Korn N. Nested PCR for specific detection and rapid identification of human picornaviruses. J Clin Microbiol 1994;32:285–91.

Kapikian AZ, Conant RM, Chanock RM, Chapple PJ, Dick EC, Fenters JD, et al. Rhinoviruses: a numbering system. Nature 1967;213:761–2.

Kelter A, Hamparian VV, Hilleman MR. Characterization and classification of ECHO 28-rhinovirus-coryzavirus agents. Proc Soc Exp Biol Med 1962;108:821–31.

Khan AG, Pichler J, Rosemann A, Blaas D. Human rhinovirus type 54 infections in polar isolation and in England. J Hyg 1989;103:196:817–25.
Kistler A, Webster DR, Rouskin S, Magrini V, Credle J, Schnurr D, et al. Genome-wide diversity and selective pressure in the human rhinovirus. Virol J 2007b;4:40.

Kling S, Donninger H, Williams Z, Vermeulen J, Weinberg E, Latiff K, et al. Persistence of rhinovirus RNA after asthma exacerbation in children. Clin Exp Allergy 2005;35:672–8.

Kolakar PR, Bella J, Olson NH, Bator CM, Baker TS, Rossman MG. Structural studies of two rhinovirus serotypes complexed with fragments of their cellular receptor. EMBO J 1999;18:6249–59.

Krilo L, Pierik L, Keller E, Mahan K, Watson D, Hirsch M, et al. The association of rhinoviruses with lower respiratory tract disease in hospitalized patients. J Med Virol 1986;19:345–52.

Kusel MMH, de Klerk NH, Holt PG, Kebadze T, Johnston SL, Sly PD. Role of respiratory viruses in acute upper and lower respiratory tract illness in the first year of life. Pediatr Infect Dis J 2005;24:680–6.

Laine P, Blomqvist S, Savolainen C, Andries K, Hovi T. Alignment of capsid protein VP1 sequences of all human rhinovirus prototype strains: conserved motifs and functional domains. J Gen Virol 2006;87:129–38.

Laine P, Savolainen C, Blomqvist S, Hovi T. Phylogenetic analysis of human rhinovirus capsid protein VP1 and 2A protease coding sequences confirms shared genus-like relationships with human enteroviruses. J Gen Virol 2005;86:697–706.

Lambert SB, Allen KM, Druce JD, Birch CJ, Mackay IM, Carlin JB, et al. Community epidemiology of human metapneumovirus, human coronavirus NL63, and other respiratory viruses in healthy preschool-aged children using parent-collected specimens. Pediatrics 2007;120:e2920–30.

Lamson D, Renwick N, Kapoor V, Liu Z, Palacios G, Ju L, et al. MassTag polymerase-chain-reaction detection of respiratory pathogens, including a new rhinovirus genotype, that caused influenza-like illness in new york state during 2004–2005. J Infect Dis 2006;2004:14398–402.

Lambert HE, Reed SE, Tyrrell DAJ. Isolation of rhinoviruses and coronaviruses from 38 colds in adults. J Med Virol 1980;5:221–9.

Lemanske RF, James DJ, Gangnon RE, Evans MD, Li Z, Shult PA, et al. Rhinovirus illnesses during infancy predict subsequent childhood wheezing. J Allergy Clin Immunol 2005;116:571–7.

Leparc I, Aymard M, Fuchs F. Acute, chronic and persistent enterovirus and poliovirus infections: detection of viral genome by seminested PCR amplification in culture-negative samples. Mol Cell Probes 1994;8:487–95.

Levandowski RA, Weaver CW, Jackson GG. Nasal-secretion leukocyte populations determined by flow cytometry during acute rhinovirus infection. J Med Virol 1988;25:423–32.

Lidwell OM, Sommerville T. Observations on the incidence and distribution of the common cold in a rural community during 1948 and 1949. J Hyg 1951;49:365–81.

Lina B, Pozzetto B, Andreoletti L, Beguier E, Bourlet T, Dussaix E, et al. Multicenter evaluation of a commercially available PCR assay for diagnosing enterovirus infection in a panel of cerebrospinal fluid specimens. J Clin Microbiol 1996;34:3002–6.

Linder JA. Improving care for acute respiratory infections: better systems, not better microbiology. Clin Infect Dis 2007;45:1189–91.

Loens K, Goossens H, de Laat C, Fioelen H, Oudshoorn P, Pattyn S, et al. Detection of rhinoviruses by tissue culture and two independent amplification techniques, nucleic acid sequence-based amplification and reverse transcription-PCR, in children with acute respiratory infections during a winter season. J Clin Microbiol 2006;44:166–71.

Lu X, Holloway B, Dare RK, Kuyers J, Yagi S, Williams JV, et al. Real-time reverse transcription-PCR assay for comprehensive detection of human rhinoviruses. J Clin Microbiol 2008;46:533–9.

Mackay IM, McErlean P, Arden KE, Faux CE, Lambert SB, Nissen MD, et al. Rhinoviruses: support act or main event? In: Schildgen O, editor. Role of newly detected and emerging viruses in childhood respiratory diseases. Nova science publishers, Inc., in press-a.

Mackay IM, Arden KE, Nissen MD, Sloots TP. Challenges Facing Real-time PCR Characterization of Acute Respiratory Tract Infections Chapter 8. In: Mackay IM editor. Real-Time PCR in Microbiology: From Diagnosis to Characterization. Caister Academic Press, pp 269–318, in press-b.

Mackay IM. Human bocavirus; multisystem detection raises questions about infection. J Infect Dis 2007;196:968–70.

Mackay IM, Bustin S, Andrade JM, Nissen MD, Sloots TP. Quantification of microorganisms: not human, not simple, not quick. In: Mackay IM, editor. Real-time PCR in microbiology. Norfolk: Caister Academic Press; 2007. p. 133–82.

Macnaughton MR. The structure and replication of rhinoviruses. Curr Topics Microbiol Immunol 1982;97:1–26.

Mahony J, Chong S, Merante F, Yaghoubian S, Sinha T, Lisle C, et al. Development of a respiratory virus panel test for detection of twenty human respiratory viruses by use of multiplex, PCR and a fluid microbead-based assay. J Clin Microbiol 2007;45:2965–70.

Mallick P, Johnston SL. How viral infections cause exacerbation of airway diseases. Chest 2006;130:1203–10.

Mallick P, Message SD, Kebadze T, Parker HL, Kon OM, Johnston SL. An experimental model of rhinovirus induced chronic obstructive pulmonary disease exacerbations: a pilot study. Respir Res 2006;7:116.

Martin JG, Siddiqui S, Hassan M. Immune responses to viral infections: Relevance for asthma. Paediatr Respir Rev 2006;7S:S125–7.

Martinez FD. Gene-environment interactions in asthma. Proc Am Thorac Soc 2007;4:26–31.

McErlean P, Shackleton LA, Andrews E, Webster DR, Lambert SB, Nissen MD, et al. Distinguishing molecular features and clinical characteristics of putative new rhinovirus species, human rhinovirus C (HRV C). PLoS Pathog 2008;4(4):e104.

McErlean P, Shackleton LA, Lambert SB, Nissen MD, Sloots TP, Mackay IM. Characterisation of a newly identified human rhinovirus, HRV-QPM, discovered in infants with bronchiolitis. J Clin Virol 2007;39:67–75.

McFadden Jr ER. Acute severe asthma. Am J Respir Crit Care Med 2003;168:740–59.

Milam DF, Smillie WG. A bacteriological study of “colds” on an isolated tropical island (St John, United States Virgin Islands, West Indies). J Exp Med 1931;53:733–52.

Miller DL, McDonald JG, Williams REO, Wilson JS. A trial of bacterial vaccines for the common cold in the Royal Air Force. Lancet 1960: 358–60.
Miller EK, Lu X, Erdman DD, Poehling KA, Zhu Y, Griffin MR, et al. Rhinovirus-associated hospitalizations in young children. J Infect Dis 2007;195:773–81.

Minor TE, Baker JW, Dick EC, DeMeo AN, Ouellette JJ, Cohen M, et al. Greater frequency of viral; respiratory infections in asthmatic children as compared with their nonasthmatic siblings. J Pediatr 1974a;85:472–7.

Minor TE, Dick EC, DeMeo AN, Ouellette JJ, Cohen M, Reed CE. Viruses as precipitants of asthmatic attacks in children. J Am Med Assoc 1974b;227:292–8.

Minor TE, Dick EC, Peterson JA, Dochtery DE. Failure of naturally acquired rhinovirus infections to produce temporal immunity to heterologous serotypes. Infect Immun 1974c;10:1192–3.

Mizgerd JP. Lung infection—a public health priority. PLoS Med 2006;3:e76.

Mogabgab WJ, Pelon W. Problems in characterizing and identifying an apparently new virus found in association with mild respiratory disease in recruits. Ann NY Acad Sci 1957;67:403–12.

Monto AS. The seasonality of rhinovirus infection and its implications for clinical recognition. Clin Therapeut 2002;24:1987–97.

Monto AS, Bryan ER, Ohmit S. Rhinovirus infections in Tecumseh, Michigan: frequency of illness and number of serotypes. J Infect Dis 1987;156:43–9.

Mori J, Clewley JP. Polymerase chain reaction and sequencing for typing rhinovirus RNA. J Med Virol 1994;44:323–9.

Nicholson KG, Kent J, Ireland DC. Respiratory viruses and exacerbations of asthma in adults. Br Med J 1993;307:982–6.

Paton W. Classification of the “2060” virus as ECHO 28 and further study of its properties. Am J Hyg 1961;73:36–54.

Paton W, Mogabgab WJ, Phillips LA, Pierce WE. A cytopathogenic agent isolated from naval recruits with mild respiratory illnesses. Proc Soc Exp Biol Med 1957;94:262–7.

Peltola V, Waris M, Österback R, Susi P, Ruuskanen O, Hyytiä T. Rhinovirus transmission within families with children: incidence of symptomatic and asymptomatic infections. J Infect Dis 2008;197:382–9.

Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, et al. UCSF chimera–A visualization system for exploratory research and analysis. J Comput Chem 2004;25:1605–12.

Pevear DC, Fancher MJ, Felock PJ, Rossmann MG, Miller MS, Diana G, et al. Conformational change in the floor of the human rhinovirus canyon blocks adsorption to HeLa cell receptors. J Virol 1989;63:2002–7.

Phillips CA, Melnick JL, Grim CA. Rhinovirus infections in a student population: Isolation of the five new serotypes. Am J Epidemiol 1968;87:447–56.

Pitkaranta A, Arruda E, Malmberg H, Hayden FG. Detection of rhinovirus in sinus brushings of patients with acute community-acquired sinusitis by reverse transcription-PCR. J Clin Microbiol 1997;35:1791–3.

Pitkaranta A, Roivainen M, Blomgren K, Peltola J, Kaijalainen T, Rätty R, et al. Presence of viral and bacterial pathogens in the nasopharynx of otitis-prone children. A prospective study. Int J Pediatr Otorhinol 2005;70:647–54.

Pitkaranta A, Virolainen A, Jero J, Arruda E, Hayden FG. Detection of rhinovirus, respiratory syncytial virus and coronavirus infections in acute otitis media by reverse-transcriptase polymerase chain reaction. Pediatr 1998;102:291–5.

Pizzichini MMM, Pizzichini E, Efthimiadis A, Chauhan AJ, Johnston SL, Hussack P, et al. Asthma and natural colds. Am J Respir Crit Care Med 1998;158:1178–84.

Price WH. The isolation of a new virus associated with respiratory clinical disease in humans. Proc Natl Acad Sci USA 1956;42:892–6.

Racanelli VR. Picornaviridae: the viruses and their replication. In: Knipe DM, Howley PM, editors. Fields virology, vol. 1. Philadelphia: Lippincott-Raven; 2001. p. 685–722.

Rakes GP, Arruda E, Ingram JM, Hoover GE, Zambrocn JO, Hayden FG, et al. Rhinovirus and respiratory syncytial virus in wheezing children requiring emergency care. Am J Respir Crit Care Med 1999;159:785–90.

Rathe J. Resolution of all known HRV-A and HRV-B complete genome sequences, and their relationship to a potential new species, HRV-C. XV European Study Group on the Molecular Biology of Picornaviruses meeting. EUROPIV 2008.

Rawlinson WD, Waluzzaman Z, Carter JW, Belessis YC, Gilbert KM, Morton JR. Asthma exacerbations in children are associated with rhinovirus but not human metapneumovirus infection. J Infect Dis 2003;187:1314–8.

Read SJ, Jeffery KJM, Bangham CRM. Aseptic meningitis and encephalitis: the role of PCR in the diagnostic laboratory. J Clin Microbiol 1997;35:691–6.

Reilly CM, Hoch SM, Stokes J, McClelland L, Hamparian VV, Ketterl A, et al. Clinical and laboratory findings in cases of respiratory illness caused by coryaviruses. Ann Intern Med 1962;57:515–25.

Renwick N, Schweiger B, Kapoor V, Liu Z, Villari J, Bullmann R, et al. Greater frequency of viral respiratory infections in asthmatic children as compared with their nonasthmatic siblings. J Pediatr 1974a;85:472–7.

Richard N, Komurian-Pradel F, Javouhey E, Perret M, Rajoharison A, Bagard G, et al. Clinical and laboratory findings in cases of respiratory illness caused by coryaviruses. Ann Intern Med 1999;159:785–90.

Rolle R, Alferi A, Fattorini R, Pasqualin M, Coletti V, et al. Association of rhinovirus infection with increased disease severity in acute bronchiolitis. Am J Respir Crit Care Med 2002a;165:1285–9.

Rolle R, Alferi A, Fattorini R, Pasqualin M, Coletti V, et al. Association of rhinovirus infection with increased disease severity in acute bronchiolitis. Am J Respir Crit Care Med 2002b;165:1285–9.
emphasis on infections by rhinovirus types 1A, 2, and two unclassified rhinoviruses. Am J Epidemiol 1971;93:183–93.

Rosenstein N, Phillips WR, Gerber MA, Marcy SM, Schwartz B, Dowell SF. The common cold – principles of judicious use of antimicrobial agents. Pediatrics 1998;101:181–4.

Rossmann MG. Viral cell recognition and entry. Protein Science 1994;3:1712–25.

Rossmann MG, Arnold E, Erickson JW, Frankenberger EA, Griffith JP, Hecht H-J, et al. Structure of a human common cold virus and functional relationship to other picornaviruses. Nature 1985;317:145–55.

Rossmann MG, Bella J, Kolakar PR, He Y, Wimmer E, Kuhn RJ, et al. Cell recognition and entry by rhino- and enteroviruses. Virology 2000;269:239–47.

Rossmann MG, He Y, Kuhn RJ. Picornavirus-receptor interactions. Trends Microbiol 2002;10:324–31.

Rotbart HA. Treatment of picornavirus infections. Antiviral Res 2002;53:83–98.

Rotbart HA, Hayden FG. Picornavirus infections: a primer for the practi- tioner. Arch Family Med 2000;9:913–20.

Rueckert RR, Wimmer E. Systematic nomenclature of picornavirus proteins. J Virol 1984;50:957–9.

Ryan J, Tucker SP, Luttick A, Hamilton S, Nearn RH. A new oral rhinovirus inhibitor BTA798. In: 18th International Conference proceedings thereof on Antiviral Research, 2005.

Savolainen C, Blomqvist S, Mulders MN, Hovi T. Genetic clustering of all 102 human rhinovirus prototype strains: serotype 87 is close to human enterovirus 70. J Gen Virol 2002;83:333–40.

Savolainen C, Laine P, Mulders MN, Hovi T. Sequence analysis of human rhinoviruses in the RNA-dependent RNA polymerase coding region reveals within-species variation. J Gen Virol 2004;85:2271–7.

Schwartz B, Marcy SM, Phillips WR, Gerber MA, Dowell SF. Pharyngitis – principles of judicious use of antimicrobial agents. Pediatrics 1998;101:171–4.

Seemungal T, Harper-Owen R, Bhownik A, Moric I, Sanderson G, Message S, et al. Respiratory viruses, symptoms, and inflammatory markers in acute exacerbations and stable chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2001;164:1618–23.

Sethi SK. Reproducible plaquing system for rhinovirus serotypes in HeLa cells – agarose suspension. Acta Virol 1978:22:60–5.

Shamnukh S, Jones L, Driskell J, Zhao Y, Dhuly R, Tripp RA. Rapid and sensitive detection of respiratory virus molecular signatures using a silver nanorod array SERS substrate. Nano Lett 2006;6:2630–6.

Silva MJ, Ferraz C, Pissarra S, Cardoso MJ, Simões J, Vitório AB. Role of viruses and atypical bacteria in asthma exacerbations among children in Oporto (Portugal). Allergologia et Immunopathologia 2007;35:4–9.

Skern T, Sommergruber W, Blaas D, Gruendler P, Fraundorfer F, Pieler C, et al. Human rhinovirus 2: Complete nucleotide sequence and proteolytic processing signals in the capsid protein region. Nucleic Acids Res 1985;13:2111–26.

Smith MBH, Feldman W. Over-the-counter cold medications: a critical review of clinical trials between 1950 and 1991. J Am Med Assoc 1992;269:2258–63.

Soyka LF, Robinson DS, Lachant N, Monaco J. The misuse of antibiotics for treatment of upper respiratory tract infections in children. Pediatrics 1975;55:552–6.

Spence L, Brown WJ, Pyne DB, Nissen MD, Sloots TP, McCormack JG, et al. Incidence, etiology, and symptomatology of upper respiratory illness in elite athletes. Med Sci Sports Exer 2007;39:577–86.

Stanway G, Hughes PJ, Mountford RC, Minor PD, Almond JW. The complete nucleotide sequence of a common cold virus: human rhinovirus 14. Nucleic Acids Res 1984;12:7859–77.

Staunton DE, Merluzzi VJ, Rothlein R, Barton R, Marlin SD, Springer TA. A cell adhesion molecule, ICAM-1, is the major surface receptor for rhinoviruses. Cell 1989;56:849–53.

Steininger C, Aberle SW, Popow-Kraupp T. Early detection of acute rhinovirus infections by a rapid reverse transcription-PCR assay. J Clin Microbiol 2001;39:129–33.
Urquhart GED, Grist NR. Virological studies of sudden, unexplained infant deaths in Glasgow 1967–1970. J Clin Pathol 1972;25:443–6.

Urquhart GED, Stott EJ. Rhinoviraemia. Br Med J 1970;4:28–30.

van den Hoogen BG, van Doornum GJJ, Fockens JC, Cornelissen JJ, Beyer WEP, de Groot R, et al. Prevalence and clinical symptoms of human metapneumovirus infection in hospitalized patients. J Infect Dis 2003;188:1571–7.

van der Zalm MM, Uiterwaal CSPM, de Jong BM, Wilbrink B, van der Ent CK. Viral specimen collection by parents increases response rate in population-based virus studies. J Allergy Clin Immunol 2006;117:955–7.

Versteegh FGA, Weverling GJ, Peeters MF, Wilbrink B, Veenstra-van Schie MTM, van Leeuwen-Gerritsen JM, et al. Community-acquired pathogens associated with prolonged coughing in children: a prospective cohort study. Clin Microbiol Infect 2005;11:801–7.

Vesa S, Kleemola M, Blomqvist S, Takala A, Kilpi T, Hovi T. Epidemiology of documented viral respiratory infections and acute otitis media in a cohort of children followed from two to twenty-four months of age. Pediatr Infect Dis J 2001;20:574–81.

Wald ER, Dashefsky B, Byers C. Frequency and severity of infection in day care. J Pediatr 1988;112:540–6.

Wald TG, Shult P, Krause F, Miller BA, Drinka P, Gravenstein S. A rhinovirus outbreak among residents of a long-term care facility. Ann Intern Med 1993;123:588–93.

Wang D, Coscoy L, Zylberberg M, Avila PC, Boushey HA, Ganem D, et al. Microarray-based detection and genotyping of viral pathogens. Proc Natl Acad Sci USA 2002;99:15687–92.

Wang QM, Chen SH. Human rhinovirus 3C protease as a potential target for the development of antiviral agents. Curr Protein Peptide Sci 2007;8:19–27.

Wark PAB, Bucchieri F, Johnston SL, Gibson PG, Hamilton L, Mimica J, et al. IFN-g-induced protein 10 is a novel biomarker of rhinovirus-induced asthma exacerbations. J Allergy Clin Immunol 2007;120:586–93.

Wark PAB, Johnston SL, Bucchieri F, Powell R, Puddicombe S, Laz-Stanca V, et al. Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. J Exp Med 2005;201:937–47.

Winther B, Gwaltney Jr JM, Mygind N, Turner RB, Hendley JO. Sites of rhinovirus recovery after point inoculation of the upper airway. J Am Med Assoc 1986;256:1763–7.

Winther B, Hayden FG, Hendley JO. Picornavirus infections in children diagnosed by RT-PCR during longitudinal surveillance with weekly sampling: association with symptomatic illness and effect of season. J Med Virol 2006;78:644–50.

Winther B, McCue K, Ashe K, Rubino JR, Hendley JO. Environmental contamination with rhinovirus and transfer to fingers of healthy individuals by daily life activity. J Med Virol 2007;79:1606–10.

Wright PF, Deatly AM, Karron RA, Belshe RB, Shi JR, Gruber WC, et al. Comparison of detection of rhinovirus by polymerase chain reaction and viral culture in human nasal wash specimens from subjects with and without clinical symptoms of respiratory illness. J Clin Microbiol 2007;45:2126–9.

Xatzipsalti M, Kyrana S, Tsolia M, Psarras S, Bossios A, Laz-Stanca V, et al. Rhinovirus viremia in children with respiratory infections. Am J Respir Crit Care Med 2005;172:1037–40.

Yoo J, Tcheurekdjian H, Lynch SV, Cabana M, Boushey HA. Microbial manipulation of immune function for asthma prevention. Inferences from clinical trials. Proceedings of the American Thoracic Society 2007;4:277–82.