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Severity of rhinovirus infection in hospitalized adults is unrelated to genotype

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An abstract of
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Abstract

Severity of rhinovirus infection in hospitalized adults is unrelated to genotype
By Denise McCulloch

Background: Differences in the severity of clinical illness due to rhinovirus (RV) species type (A, B or C) have been found in pediatric populations. In adults, however, the relationship between RV species and clinical illness is less well characterized.

Objectives: To determine whether RV species is associated with more severe clinical illness in adults.

Study design: Seventy-two RV-positive viral respiratory samples from adult patients were sequenced and analyzed phylogenetically. The clinical features and severity of illness were compared for the different RV species using ANOVA. Logistic regression was used to model the relationship between RV type and severity of illness.

Results: Phylogenetic analysis identified three distinct clusters as RV-A (54%), B (19%) or C (26%) species. The groups were demographically similar except that in the RV-C group, there were more females ($p=0.05$), and in the RV-B group, a larger proportion of patients had diabetes ($p=0.04$). In an unadjusted model, patients with RV-B infection as compared to patients with RV-C infection were significantly more likely to have the composite outcome variable of death or ICU admission ($p=0.03$), but this effect diminished when controlling for patient sex. A logistic model of the relationship between RV species and adverse outcomes produced nonsignificant odds ratios when controlling for patient sex.

Conclusions: Infection with RV-A or RV-B was associated with greater severity of illness in an adult population; however, the association disappeared after multivariate analysis controlling for the confounding effect of gender.

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Background

Introduction

Rhinoviruses are best known for causing the common cold; indeed, they are responsible for the majority of non-influenza-related viral respiratory tract infections (1, 2). In spite of the relatively low morbidity associated with most of these infections, however, they are responsible for $17 billion in direct health-care costs and $22 billion in indirect costs each year in the United States (3). Moreover, the clinical impact of rhinovirus infections is not limited to their role in causing the common cold; rhinoviruses have been implicated in acute otitis media, sinusitis, and lower respiratory tract disease (1, 4-6). Furthermore, viral upper respiratory infections, which are most commonly attributable to rhinovirus, cause up to 80% of pediatric asthma exacerbations and half of adult asthma and chronic obstructive pulmonary disease (COPD) exacerbations (7, 8).

Rhinoviruses have been identified as precipitants of asthma exacerbations – first, in observational studies (9) and subsequently in experimentally induced rhinovirus infection (10, 11). Later studies revealed that rhinoviruses not only play a role in exacerbating reactive airway disease, but also may play a role in its pathogenesis (12, 13) via upregulation of proinflammatory mediators and airway remodeling (14-19).

In children, the relationship between rhinovirus infection and asthma exacerbation is modified by the particular species of rhinovirus responsible for the respiratory infection (20). The relationship between rhinovirus species and clinical illness, however, has yet to be fully defined.

Rhinovirus Virology and Taxonomy

Rhinoviruses (RV) are non-enveloped, single-stranded RNA viruses belonging to the *Enterovirus* genus of the family *Picornaviridae*. Recently the International Committee on Taxonomy of Viruses changed the nomenclature to remove host species from picornavirus species names, and the species names are currently known as *Rhinovirus A*, *B* and *C* instead of *Human rhinovirus A*, *B* and *C* (21).

Sequencing of rhinovirus genomes has identified three distinct RV species, RV-A (77 types), RV-B (25 types), and RV-C species (50 types) (22, 23). The term 'type' has replaced serotype, and RV-C species has been described by sequencing, as there is no antigenic typing for RV-C. RV-C has been described in some literature as HRV-A2 (24, 25), HRV-C (26-29), or HRV-X(30) but in this study will all be referred to as RV-C (31, 32). The >150 RV types differ in the amino acid sequences of their viral capsid proteins, resulting in antigenic variation (6, 33). Despite this antigenic diversity all known RV-A and RV-B types bind only two known cell surface receptors and are grouped accordingly. Major group RVs bind to the intracellular adhesion molecule 1 (ICAM-1) (34) while minor-group RVs bind the low-density lipoprotein receptor (35). These receptors enable virus entry through the host cell membrane, and are therefore also important as potential targets for therapeutic intervention. The receptor for RV-C has not been identified but is thought to be distinct from the receptors for RV-A and RV-B (1, 36).

The third species of rhinovirus, RV-C, was first described in 2007 (5, 25, 27, 28, 30, 37). Though a newly discovered type, evidence suggests RV-C is not a new or emerging virus, but rather, one that earlier techniques had been unable to identify (37-40). The epidemiologic literature suggests that the virus has global, year-round

circulation, with some studies reporting peak RV-C prevalence in the winter months (25, 27, 39). The reported frequency of RV-C detection varies widely across studies, ranging from 11—89% (Appendix A). Most commonly, however, its prevalence lies between 30-50%, in between that of RV-A, the most common species, and RV-B, the least common (27).

Rhinovirus species and severity of clinical illness

Early studies of the newly identified rhinovirus species RV-C sought, first, to molecularly characterize the novel RV strain, and second, to identify the range of clinical illnesses with which it is associated. These studies revealed that RV-C is associated with a broad range of clinical manifestations, from asymptomatic colonization to upper and lower respiratory tract infection (41, 42), wheezing (43, 44), asthma exacerbation (45), bronchitis (37), bronchiolitis (25, 46), pneumonia (36, 47), and pericarditis (48).

Studies directly comparing the clinical effects of one RV species versus another have for the most part been conducted in pediatric populations and have produced highly variable results (Appendix B). Most commonly, RV-C has been associated with severe illness. Eight studies found evidence of more severe disease in patients with RV-C, as manifested by lower respiratory tract infection (39), oxygen requirement (49, 50), viremia (51), pneumonia (36), hospital admissions (52), ICU admissions (53) and asthma severity scores (20).

Clouding the picture somewhat, three studies have concluded that RV-A and C are associated with more severe illness than RV-B (45, 54, 55), while three other studies

found that RV-A and B were associated with more severe illness than RV-C (42, 56, 57). Two studies found RV-B to be associated with more severe illness (46, 58).

A number of negative studies have been published as well, however—five studies, four of which involved exclusively pediatric patients, have found no difference in the clinical manifestations of the three RV species (27, 43, 59-61).

Of the twenty-two studies described here, only four included adult patients. Of these, two found that RV-A and RV-B were associated with severe disease (56, 57), one implicated RV-C (36), and one study found no difference at all (61).

In adults, therefore, the association between species and clinical severity remains ill-defined. One reason is that infection with rhinovirus in adults typically follows a mild course. In elderly patients, those with chronic lung disease and the immunocompromised, however, severe outcomes in adult patients have been observed (62-64).

Thus, although some studies have raised the possibility of severe disease in adults arising from particular rhinovirus species, the particular strain responsible for the most severe disease has varied, and the relationship between RV species and clinical illness in adults has yet to be defined.

Methods

Study objective

The goal of this study is to determine whether, in an adult patient population, there is a particular rhinovirus species that is specifically associated with more severe illness, as measured by surrogate markers of disease severity.

Hypothesis

Rhinovirus species C would be associated with more severe clinical illness, as indicated by higher rates of mortality and ICU admission.

Study Design

This retrospective cohort study reviewed chart and laboratory data on patients seen at Emory Healthcare from October 2009 to April 2010 whose respiratory specimens were positive for rhinovirus.

Study site and Institutional Review Board Approval

Emory Healthcare includes 4 hospitals, 2 emergency departments, and a large, multi-specialty outpatient clinic with almost entirely adult patients. Approval was obtained for retrospective chart review from the Emory University Institutional Review Board (#33341).

Study inclusion criteria

Patients were included in the study if their viral respiratory panel sample was y were positive for rhinovirus between October 2009 and April 2010. Patients younger than 18 years of age were excluded (n=3). Ten samples could not be amplified and five medical records were inaccessible; these were excluded from the analyses. Patients

whose sequencing subsequently revealed the presence of not rhinovirus but another virus were also excluded (n=8).

Laboratory testing and rhinovirus sequencing

Patient respiratory samples (nasopharyngeal swabs or bronchoalveolar lavage) in viral transport media underwent routine clinical testing for rhinovirus/enterovirus, influenza A, parainfluenza, adenovirus, metapneumovirus, and RSV by xTAG® RVP (Luminex Corporation, Austin, TX), a multiplexed nucleic acid assay that enables the simultaneous detection of multiple distinct viruses (65). Positive samples were archived at -80°C and sequenced. Seventy-two patient specimens were successfully amplified using RT-PCR and sequenced; reference sequences were used for species assignment of the samples. Details of sequencing are described elsewhere (66).

Data Collection

Electronic medical records of patients positive for rhinovirus were reviewed. The following clinical data were abstracted: age, race, gender, co-morbidities, infections at other body sites, antiviral and antibiotic therapy, length of stay (LOS), hospitalization status, intensive care unit (ICU) admission, inpatient mortality, signs and symptoms on presentation, radiographic findings, and laboratory values (white blood count, hematocrit, platelet count, AST, ALT).

Major co-morbidities were grouped by organ system and patients with actively treated malignancies, HIV infection, rheumatologic conditions on immunosuppressive therapy, or recipients of solid organ or hematopoietic transplants were considered immunocompromised. Patients were considered to have an infection at another site if

they had a bacterial, fungal, or additional viral infection from any source, or a clinical diagnosis of pneumonia or urinary tract infection at the time of respiratory testing. Antiviral therapy included treatment with oseltamivir, zanamavir or peramivir. Antibiotic therapy included any antibacterial agent given around the time of respiratory testing. Signs and symptoms were abstracted from the medical record on the day of the medical encounter that resulted in ordering the respiratory viral panel testing. Fever was defined as either a subjective, patient-reported fever or a documented temperature >37.8°C. The primary endpoint was a composite variable consisting of death during inpatient stay and/or admission to the ICU because these individual outcomes were infrequent.

Statistical analysis

All analyses were performed using SAS 9.3 (Cary, NC). *P*-values of <0.05 were considered to be statistically significant. Using a normal approximation to calculate power, enrolment of 72 patients was found to provide a power of 80% to detect a 34% risk difference in the incidence of death or ICU admission in RV-A plus RV-B group, as compared with the RV-C group (67).

Univariate analyses were performed and variables that followed an approximately normal distribution were treated as numeric variables in subsequent analyses. Variables that did not follow a normal distribution were converted into categorical variables for analysis. Numerical variables were compared using Student's t-test for two-sample comparisons and ANOVA for comparisons between more than two groups. Categorical variables were compared using chi-square or Fisher's exact test, where appropriate.

Analysis for identification of confounders

For each of the demographic and clinical factors measured in the study, a t-test for continuous variables and a chi square test for categorical variables were used to assess whether the factor was significantly associated with RV species and with the outcome variables; factors which were significantly associated with both were identified as potential confounders.

Interaction

Interaction terms were created and included in the logistic regression models to assess for potentially significant interactions between the variables and risk factors under study.

Regression

Logistic regression was performed to model the relationship between rhinovirus species and a composite primary endpoint of death or ICU admission. This model was used to generate odds ratios (OR) with 95% confidence intervals (CI) for the study population, first as an unadjusted model, and subsequently in a model adjusting for confounders.

Results

Excluded Subjects

Subjects from whom no viral sequences were amplified by RT-PCR were excluded from analysis. Of the 98 total samples, 10 (10%) were not typed. Compared to included subjects, those who were excluded because their samples could not be amplified were significantly more likely to have diabetes (p=0.05) and less likely to have hypertension (p=0.01). Across other demographic and clinical characteristics, patients whose samples were not able to be amplified were not significantly different from those who were included in the study. The primary outcome measures of death and ICU admission also did not differ between subtyped and non-typed (excluded) patients (p=0.22 and p=0.34, respectively.)

Results of phylogenetic analysis

Of 2261 viral respiratory panel samples analyzed between October of 2009 and April 2010, 105 were positive for rhinovirus. After exclusions, 72 patients were included in the final analysis. Eighty-eight discrete patient samples underwent sequencing and were determined by phylogenetic analysis to be RV-A (n=39), RV-B (n=8) or RV-C (n=25) (Figure 1). RV typing beyond species assignment was not attempted, but there were clusters of infections caused by very closely related, single RV types in the species clades for RV-A and RV-C. This is consistent with RV sampling over a single season from a single geographic location.

Demographic and Clinical Characteristics (Table 1)

The majority of patients were hospitalized (64%). The mean age was 47 years (range, 18-82); 53% were female, 30% had an underlying diagnosis of asthma, bronchitis,

COPD or other pulmonary comorbidity, and 19% had bacterial pneumonia as diagnosed by the clinical team. Forty-nine percent were immunocompromised, of whom 17% were HIV-positive and 54% were solid organ transplant recipients. Hypertension (42%), diabetes (13%) and malignancy (30%) were also common. The proportion of female patients was significantly different among the 3 RV groups (p=0.05); as was the proportion of patients with diabetes (p=0.04). Otherwise, the groups were demographically similar.

Rhinovirus was the only respiratory virus found in all but two of the 72 subjects. Among RV positive patients one (RV-A) also had parainfluenza and one (RV-C) had influenza A. Neither patient died nor was admitted to the ICU. None of the patients were infected with more than one type of rhinovirus.

Across all RV groups, the majority of patients had a cough and a minority had diarrhea and vomiting. The prevalence of these symptoms did not differ significantly across groups. Laboratory findings including complete blood counts and liver function tests (transaminases) were similar across groups, as was the prevalence of abnormal chest radiographs.

Results – patient outcomes – univariate analysis

The proportion of patients with a length of stay (LOS) greater than 7 days was significantly different among the RV species groups (p=0.03), with 54.9% of RV-A patients and 14.3% of RV-B patients being hospitalized for longer than 7 days (Table 2). The proportion of patients admitted to the ICU was not significantly different among RV species groups, nor was the proportion of patients in each group who died. The proportion of patients whose outcome was death or ICU admission was significantly

different among RV species (p=0.04): this outcome occurred in 38% percent of RV-B patients, 21% of RV-A patients and 4% of RV-C patients.

Assessment of potential confounders

The only factor that was significantly associated with both RV type and outcome measures at the 0.05 alpha level was patient sex (Table 3).

Results—logistic model

The unadjusted logistic model of the relationship between RV species and clinical disease severity demonstrated that the odds of death or ICU admission among patients with RV-B infection was 15 times that of patients with RV-C infection (OR = 15.0, 95% CI 1.3—175.3, p=0.03). RV-C infection was chosen as the reference group given our hypothesis that RV-C would be associated with more severe illness than RV-A and RV-B. For RV-A, the odds ratio was not significant. An adjusted model (Table 4) was constructed, controlling for patient sex, which was identified as the only variable associated with both the RV species and the outcome measure. In the adjusted model, the relationship between RV-A (OR = 4.4, 95% CI: 0.5—39.8) or RV-B (OR = 12.2, 95% CI: 0.994—15.0) and the composite outcome measure of death or ICU admission was not significant at the 0.05 alpha level.

Death and/or ICU admission in these patients did not result exclusively from respiratory illness; these outcomes tended to be multifactorial. Inclusion of other determinants of health status, however, did not change the results of the model of the relationship between RV subtype and adverse outcome. Additional models were constructed to evaluate the contribution of comorbidities to the outcome; in these models, none were significant (Table 5).

Additional models were constructed to further characterize the relationship between RV type and severity of illness. Specifically, models were constructed to include interaction terms for assessment of potential interaction between RV subtype and patient sex, immunocompromised status, pulmonary comorbidities and significant coinfections; of these, none were significant.

Results—immunocompromised patients and patients with pulmonary comorbidities

No RV species was associated with greater clinical severity in analyses of sub-populations with pulmonary comorbidities (primarily asthma and COPD). Pulmonary comorbidities were also not independently associated with increased likelihood of severe illness. Additional analyses examining the relationship between RV and severity of clinical illness in immunocompromised patients found no significant relationship between RV species and severity of clinical illness. The relationship between immunocompromised status and severity of illness was also not significant (data not shown).

Discussion

This study has described the relationship between RV species and severity of clinical illness in an adult population. In unadjusted pairwise comparisons, RV-A and RV-B were associated with greater severity of illness compared with RV-C infection, as measured by the composite proxy outcome measure of ICU admission or death. An analysis of potential confounders, considering patient sex, age, immunocompromised status, co-infection, pneumonia, and malignancy identified only sex as significantly associated with both RV species and the outcomes of interest. Female sex was significantly associated both with severe disease and with RV species and was thus identified as a confounder that should be included in the adjusted model. When patient sex was included in the logistic model, the relationship between RV-A and RV-B and more severe disease was no longer significant.

The key question that emerges from these findings is whether the lack of association that emerged after controlling for other variables reflects a true null result or whether the small sample size in this study limited ability to detect an existing difference. Although this study had 80% power, this leaves a nontrivial 20% chance of Type II error. Furthermore, the paucity of existing data on clinical characterization of RV infection in adults means that the estimate from the literature (56) of a 34% difference among groups may predict a greater risk difference than could be confirmed in this dataset.

Previous analysis of RV species and severity of clinical illness in adults has found that RV-A and RV-B species were associated with influenza-like illness among adult patients but RV-C was not (56, 57). The significant findings from unadjusted model (Table 3), where RV-A and RV-B were associated with more severe disease than RV-C,

are consistent with these previous studies. In the logistic model controlling for patient sex, however, the estimated odds ratios for the relationship between RV species and clinical outcome were no longer significant. Of note, these odds ratio remained in the positive direction, and for RV-B, the p-value was 0.051. It is therefore quite plausible that, given a larger sample size, the statistically significant association between these species and more severe illness would have persisted even after controlling for potential confounders.

Sex differences in cellular immunity to RV have been described in the literature and are thought to be related to hormonal influences on the immune system (68). Whether this difference in immunity translates into differences in clinical outcomes, however, is unclear. In this study, patient sex was significantly associated with both RV species and the primary composite outcome variable of death or ICU admission. Including sex in the logistic model produced a marginally significant odds ratio of 3.4 for the effect of patient sex on adverse outcomes ($p=0.10$). That is, the odds of adverse outcome (ICU admission or death) are 3.4 times higher for female patients than for male patients. This lends support to the idea that differences in the adaptive immune response to RV infection could in fact translate to dissimilar clinical outcomes in men and women.

Sex differences in the immune response to RV infection also have potential implications for the role of RV infection in the pathogenesis of reactive airway disease. If rhinovirus contributes to the development of asthma via the immune/inflammatory response it provokes, and women have a stronger response, perhaps RV infection contributes to sex differences in asthma prevalence. From puberty onward, the incidence,

prevalence and severity of asthma are greater in females than in males; hormonal factors are thought to influence airway hyperresponsiveness (69).

Strengths and Limitations

The major limitation in this study was the relatively small sample size, resulting in only 80% power to detect a 34% risk difference between two groups. In particular, the small number of patients in the RV-B group limits the ability to draw conclusions about patients with RV-B infection.

A second limitation of this study is that the mostly hospitalized patient population had such a plethora of comorbidities that controlling for every single one of them was impossible. Therefore, although included a large number of potential confounders in logistic models, it is possible that other potentially important factors eluded us.

Furthermore, in these often very ill patients, with a large number of simultaneous pathologies and diagnoses, teasing apart the role of each pathogen or disease is difficult. Although tried to control for this to the greatest possible extent, isolating the individual contribution of rhinovirus in a complex medical case is difficult.

One strength of this study is its contribution to an area in which little is known. literature review identified twenty-two studies of the relationship between rhinovirus species and clinical illness; of these, only two included adult patients. Both studies addressed patient symptoms, but neither addressed treatment nor outcomes (56, 57). In spite of its small sample size, therefore, this study makes some early steps towards better understanding of this relationship in adult patients.

Second, although ten respiratory samples could not be amplified, and the patients were therefore excluded from the study, analyzed all of the excluded patients'

demographic and clinical characteristics. These were compared to those of included patients and found to be very similar, helping to reduce concerns about possible sampling bias that might have resulted from exclusion of these patients.

Third, the thorough chart review process resulted in ample clinical data about each patient, and allowed to control for a large number of potential confounders in analysis. Furthermore, testing for a number of different respiratory viruses and examination of specific patients with co-infections helped isolate, to the best possible extent, the contribution of rhinovirus infection.

Conclusion

This study elucidates some of the unique aspects of the molecular and clinical epidemiology of RV infection in adult populations and highlights some important differences from the pediatric literature, in which RV-C infection has been associated with more severe illness. This study's findings suggest that in adults with RV infection, species-dependent differences in clinical outcomes are not the same as in children, and may not exist at all. If these differences do exist in adults, however, they may be related to patients' sex and associated immunologic and hormonal differences.

Implications

Attempts to "cure the common cold" have thus far proven fruitless—to date, efforts at creating a vaccine or antiviral medication have not been successful (70). This is in part due to the vast number of rhinovirus serotypes and lack of data about the most commonly circulating RV strains (71). If one species of RV were identified as the primary culprit

for severe illness, however, this could help direct future therapeutic initiatives towards the most virulent species. Given the tremendous frequency and cost of rhinovirus infections each year, as well as the potential for severe illness in vulnerable populations, advances in preventive and therapeutic treatments could have tremendous public health impacts.

Future Directions

Two advances in laboratory characterization of rhinovirus will be critical in advancing understanding and treatment of infection. First, identification of the RV-C receptor may be a key step towards developing antiviral vaccines and treatments. Second, development of a rapid, point-of-care test for rhinovirus could help providers distinguish rhinovirus infection from bacterial respiratory infection, helping to reduce unnecessary antibiotic use.

From an epidemiologic perspective, larger studies, conducted over multiple seasons, and incorporating a mix of relatively healthy outpatients as well as hospitalized patients with multiple comorbidities, will be needed to help clarify the true relationship of RV species and clinical illness in adult patients. This will be particularly useful in patients with underlying respiratory illness, such as asthma and COPD, who are already prone to exacerbation of their illness by respiratory infections, and patients who are immunocompromised, and most at risk for severe clinical illness from the infection.

References

1. Winther B. Rhinovirus infections in the upper airway. *Proc Am Thorac Soc* 2011;8(1):79-89.
2. Piralla A, Baldanti F, Gerna G. Phylogenetic Patterns of Human Respiratory Picornavirus Species, Including the Newly Identified Group C Rhinoviruses, during a 1-Year Surveillance of a Hospitalized Patient Population in Italy. *J Clin Microbiol* 2011;49(1):373-6.
3. Fendrick AM, Monto AS, Nightengale B, et al. The economic burden of non-influenza-related viral respiratory tract infection in the United States. *Arch Intern Med* 2003;163(4):487-94.
4. van Piggelen RO, van Loon AM, Krediet TG, et al. Human rhinovirus causes severe infection in preterm infants. *The Pediatric infectious disease journal* 2010;29(4):364-5.
5. Kiang D, Yagi S, Kantardjieff KA, et al. Molecular characterization of a variant rhinovirus from an outbreak associated with uncommonly high mortality. *J Clin Virol* 2007;38(3):227-37.
6. Henquell C, Mirand A, Deusebis AL, et al. Prospective genotyping of human rhinoviruses in children and adults during the winter of 2009-2010. *J Clin Virol* 2012;53(4):280-4.
7. Friedlander SL, Busse WW. The role of rhinovirus in asthma exacerbations. *J Allergy Clin Immunol* 2005;116(2):267-73.
8. McManus TE, Marley AM, Baxter N, et al. Respiratory viral infection in exacerbations of COPD. *Respir Med* 2008;102(11):1575-80.
9. Minor TE, Dick EC, Baker JW, et al. Rhinovirus and influenza type A infections as precipitants of asthma. *The American review of respiratory disease* 1976;113(2):149-53.
10. Halperin SA, Eggleston PA, Beasley P, et al. Exacerbations of asthma in adults during experimental rhinovirus infection. *The American review of respiratory disease* 1985;132(5):976-80.
11. Lemanske RF, Jr., Dick EC, Swenson CA, et al. Rhinovirus upper respiratory infection increases airway hyperreactivity and late asthmatic reactions. *The Journal of clinical investigation* 1989;83(1):1-10.
12. Proud D. Role of rhinovirus infections in asthma. *Asian Pacific journal of allergy and immunology / launched by the Allergy and Immunology Society of Thailand* 2011;29(3):201-8.
13. Jackson DJ, Evans MD, Gangnon RE, et al. Evidence for a causal relationship between allergic sensitization and rhinovirus wheezing in early life. *Am J Respir Crit Care Med* 2012;185(3):281-5.
14. Tacon CE, Wiehler S, Holden NS, et al. Human Rhinovirus Infection Upregulates MMP-9 Production in Airway Epithelial Cells via NF-{kappa}B. *Am J Respir Cell Mol Biol* 2009.
15. Skevaki CL, Psarras S, Volonaki E, et al. Rhinovirus-induced basic fibroblast growth factor release mediates airway remodeling features. *Clinical and translational allergy* 2012;2(1):14.

16. Kuo C, Lim S, King NJ, et al. Rhinovirus infection induces extracellular matrix protein deposition in asthmatic and nonasthmatic airway smooth muscle cells. *American journal of physiology Lung cellular and molecular physiology* 2011;300(6):L951-7.
17. Kuo C, Lim S, King NJ, et al. Rhinovirus infection induces expression of airway remodelling factors in vitro and in vivo. *Respirology* 2011;16(2):367-77.
18. Miller EK. New human rhinovirus species and their significance in asthma exacerbation and airway remodeling. *Immunol Allergy Clin North Am* 2010;30(4):541-52, vii.
19. Leigh R, Oyelusi W, Wiehler S, et al. Human rhinovirus infection enhances airway epithelial cell production of growth factors involved in airway remodeling. *J Allergy Clin Immunol* 2008;121(5):1238-45 e4.
20. Bizzintino J, Lee WM, Laing IA, et al. Association between human rhinovirus C and severity of acute asthma in children. *Eur Respir J* 2011;37(5):1037-42.
21. International Committee on Taxonomy of Viruses., King AMQ, International Union of Microbiological Societies. Virology Division. *Virus taxonomy : classification and nomenclature of viruses : ninth report of the International Committee on Taxonomy of Viruses*. Amsterdam: Elsevier/Academic Press; 2012.
22. International Committee on Taxonomy of Viruses. (http://www.picornaviridae.com/enterovirus/enterovirus.htm). (Accessed August 8 2012).
23. Palmenberg AC, Spiro D, Kuzmickas R, et al. Sequencing and analyses of all known human rhinovirus genomes reveal structure and evolution. *Science* 2009;324(5923):55-9.
24. Arden KE, McErlean P, Nissen MD, et al. Frequent detection of human rhinoviruses, paramyxoviruses, coronaviruses, and bocavirus during acute respiratory tract infections. *J Med Virol* 2006;78(9):1232-40.
25. McErlean P, Shackelton LA, Lambert SB, et al. Characterisation of a newly identified human rhinovirus, HRV-QPM, discovered in infants with bronchiolitis. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology* 2007;39(2):67-75.
26. Lamson D, Renwick N, Kapoor V, 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;194(10):1398-402.
27. Lau SK, Yip CC, Tsoi HW, et al. Clinical features and complete genome characterization of a distinct human rhinovirus (HRV) genetic cluster, probably representing a previously undetected HRV species, HRV-C, associated with acute respiratory illness in children. *J Clin Microbiol* 2007;45(11):3655-64.
28. Lee WM, Kiesner C, Pappas T, et al. A diverse group of previously unrecognized human rhinoviruses are common causes of respiratory illnesses in infants. *PLoS One* 2007;2(10):e966.
29. McErlean P, Shackelton LA, Andrews E, et al. Distinguishing molecular features and clinical characteristics of a putative new rhinovirus species, human rhinovirus C (HRV C). *PLoS One* 2008;3(4):e1847.

30. Kistler A, Avila PC, Rouskin S, et al. Pan-viral screening of respiratory tract infections in adults with and without asthma reveals unexpected human coronavirus and human rhinovirus diversity. *J Infect Dis* 2007;196(6):817-25.
31. Simmonds P, McIntyre C, Savolainen-Kopra C, et al. Proposals for the classification of human rhinovirus species C into genotypically assigned types. *J Gen Virol* 2010;91(Pt 10):2409-19.
32. Rueter K, Bizzintino J, Martin AC, et al. Symptomatic viral infection is associated with impaired response to treatment in children with acute asthma. *J Pediatr* 2012;160(1):82-7.
33. Kennedy JL, Turner RB, Braciale T, et al. Pathogenesis of rhinovirus infection. *Curr Opin Virol* 2012;2(3):287-93.
34. Greve JM, Davis G, Meyer AM, et al. The major human rhinovirus receptor is ICAM-1. *Cell* 1989;56(5):839-47.
35. Hofer F, Gruenberger M, Kowalski H, et al. Members of the low density lipoprotein receptor family mediate cell entry of a minor-group common cold virus. *Proceedings of the National Academy of Sciences of the United States of America* 1994;91(5):1839-42.
36. Lau SK, Yip CC, Lin AW, et al. Clinical and molecular epidemiology of human rhinovirus C in children and adults in Hong Kong reveals a possible distinct human rhinovirus C subgroup. *J Infect Dis* 2009;200(7):1096-103.
37. Renwick N, Schweiger B, Kapoor V, et al. A recently identified rhinovirus genotype is associated with severe respiratory-tract infection in children in Germany. *J Infect Dis* 2007;196(12):1754-60.
38. Miller EK, Mackay IM. From sneeze to wheeze: what we know about rhinovirus Cs. *J Clin Virol* 2013;57(4):291-9.
39. Linder JE, Kraft DC, Mohamed Y, et al. Human rhinovirus C: Age, season, and lower respiratory illness over the past 3 decades. *J Allergy Clin Immunol* 2013;131(1):69-77 e1-6.
40. Briese T, Renwick N, Venter M, et al. Global distribution of novel rhinovirus genotype. *Emerg Infect Dis* 2008;14(6):944-7.
41. Han TH, Chung JY, Hwang ES, et al. Detection of human rhinovirus C in children with acute lower respiratory tract infections in South Korea. *Arch Virol* 2009;154(6):987-91.
42. Jin Y, Yuan XH, Xie ZP, et al. Prevalence and clinical characterization of a newly identified human rhinovirus C species in children with acute respiratory tract infections. *J Clin Microbiol* 2009;47(9):2895-900.
43. Piotrowska Z, Vazquez M, Shapiro ED, et al. Rhinoviruses are a major cause of wheezing and hospitalization in children less than 2 years of age. *Pediatr Infect Dis J* 2009;28(1):25-9.
44. Smuts HE, Workman LJ, Zar HJ. Human rhinovirus infection in young African children with acute wheezing. *BMC infectious diseases* 2011;11:65.
45. Khetsuriani N, Lu X, Teague WG, et al. Novel human rhinoviruses and exacerbation of asthma in children. *Emerg Infect Dis* 2008;14(11):1793-6.
46. Calvo C, Casas I, Garcia-Garcia ML, et al. Role of rhinovirus C respiratory infections in sick and healthy children in Spain. *Pediatr Infect Dis J* 2010;29(8):717-20.

47. Broberg E, Niemela J, Lahti E, et al. Human rhinovirus C--associated severe pneumonia in a neonate. *J Clin Virol* 2011;51(1):79-82.
48. Tapparel C, L'Huillier AG, Rougemont AL, et al. Pneumonia and pericarditis in a child with HRV-C infection: a case report. *J Clin Virol* 2009;45(2):157-60.
49. Miller EK, Khuri-Bulos N, Williams JV, et al. Human rhinovirus C associated with wheezing in hospitalised children in the Middle East. *J Clin Virol* 2009;46(1):85-9.
50. Mak RK, Tse LY, Lam WY, et al. Clinical spectrum of human rhinovirus infections in hospitalized Hong Kong children. *Pediatr Infect Dis J* 2011;30(9):749-53.
51. Fuji N, Suzuki A, Lupisan S, et al. Detection of human rhinovirus C viral genome in blood among children with severe respiratory infections in the Philippines. *PLoS One* 2011;6(11):e27247.
52. Cox DW, Bizzintino J, Ferrari G, et al. Human rhinovirus species C infection in young children with acute wheeze is associated with increased acute respiratory hospital admissions. *Am J Respir Crit Care Med* 2013;188(11):1358-64.
53. Lauinger IL, Bible JM, Halligan EP, et al. Patient characteristics and severity of human rhinovirus infections in children. *J Clin Virol* 2013;58(1):216-20.
54. Lee WM, Lemanske RF, Jr., Evans MD, et al. Human rhinovirus species and season of infection determine illness severity. *Am J Respir Crit Care Med* 2012;186(9):886-91.
55. Xiang Z, Gonzalez R, Xie Z, et al. Human rhinovirus C infections mirror those of human rhinovirus A in children with community-acquired pneumonia. *J Clin Virol* 2010;49(2):94-9.
56. Watanabe A, Carraro E, Kamikawa J, et al. Rhinovirus species and their clinical presentation among different risk groups of non-hospitalized patients. *J Med Virol* 2010;82(12):2110-5.
57. Xiang Z, Gonzalez R, Wang Z, et al. Human rhinoviruses in Chinese adults with acute respiratory tract infection. *The Journal of infection* 2010;61(4):289-98.
58. Miller EK, Williams JV, Gebretsadik T, et al. Host and viral factors associated with severity of human rhinovirus-associated infant respiratory tract illness. *J Allergy Clin Immunol* 2011;127(4):883-91.
59. Huang T, Wang W, Bessaud M, et al. Evidence of recombination and genetic diversity in human rhinoviruses in children with acute respiratory infection. *PLoS One* 2009;4(7):e6355.
60. Iwane MK, Prill MM, Lu X, et al. Human rhinovirus species associated with hospitalizations for acute respiratory illness in young US children. *J Infect Dis* 2011;204(11):1702-10.
61. Tapparel C, Cordey S, Junier T, et al. Rhinovirus genome variation during chronic upper and lower respiratory tract infections. *PLoS One* 2011;6(6):e21163.
62. Falsey AR, Walsh EE, Hayden FG. Rhinovirus and coronavirus infection-associated hospitalizations among older adults. *J Infect Dis* 2002;185(9):1338-41.
63. Kraft CS, Jacob JT, Sears MH, et al. Severity of human rhinovirus infection in immunocompromised adults is similar to 2009 H1N1 influenza. *J Clin Microbiol* 2012.
64. Louie JK, Yagi S, Nelson FA, et al. Rhinovirus outbreak in a long term care facility for elderly persons associated with unusually high mortality. *Clin Infect Dis* 2005;41(2):262-5.

65. Merante F, Yaghoubian S, Janeczko R. Principles of the xTAG respiratory viral panel assay (RVP Assay). *J Clin Virol* 2007;40 Suppl 1:S31-5.
66. Kraft CS, Sears MH, Thurmond C, et al. Phylogenetic Analysis of Rhinoviral Sequences From Respiratory Virus Season 2009-2010. *26th Clinical Virology Symposium*. Daytona Beach, Florida, USA 2010.
67. Dean AG SK, Soe MM. OpenEpi: Open Source Epidemiologic Statistics for Public Health. (http://www.OpenEpi.com). (Accessed December 30, 2013).
68. Carroll ML, Yerkovich ST, Pritchard AL, et al. Adaptive immunity to rhinoviruses: sex and age matter. *Respir Res* 2010;11:184.
69. Sears MR. Epidemiology of asthma exacerbations. *J Allergy Clin Immunol* 2008;122(4):662-8; quiz 9-70.
70. Linder JA. Vitamin D and the cure for the common cold. *JAMA* 2012;308(13):1375-6.
71. Jacobs SE, Lamson DM, St George K, et al. Human rhinoviruses. *Clinical microbiology reviews* 2013;26(1):135-62.
72. Savolainen-Kopra C, Blomqvist S, Kaijalainen S, et al. All known human rhinovirus species are present in sputum specimens of military recruits during respiratory infection. *Viruses* 2009;1(3):1178-89.
73. Piralla A, Rovida F, Campanini G, et al. Clinical severity and molecular typing of human rhinovirus C strains during a fall outbreak affecting hospitalized patients. *J Clin Virol* 2009;45(4):311-7.
74. Wisdom A, Kutkowska AE, McWilliam Leitch EC, et al. Genetics, recombination and clinical features of human rhinovirus species C (HRV-C) infections; interactions of HRV-C with other respiratory viruses. *PloS one* 2009;4(12):e8518.

Tables

Table 1. Demographics and clinical characteristics of patients with RV infection, total and by RV species

	Total (n=72)	**RV-A** (n=39)	**RV-B** (n=8)	**RV-C** (n= 25)	**p-value**
Mean age ± SD	47±17	45±16	52±19	47±16	0.57
Female *n* (%)	38 (53)	16 (41)	4 (50)	18 (72)	0.05*
Inpatients *n* (%)	**46 (64)**	**27 (69)**	**6 (75)**	**13 (52)**	**0.02***
Comorbidities *n* (%)					
Hypertension	30 (42)	14 (36)	6 (75)	10 (40)	0.13
Diabetes	9 (13)	2 (5)	3 (38)	4 (16)	0.04*
Malignancy	22 (31)	12 (31)	3 (38)	7 (28)	0.87
Cardiac	15 (21)	6 (15)	2 (25)	7 (28)	0.49
Pulmonary	22 (31)	9 (23)	3 (38)	10 (40)	0.30
Renal	12 (17)	7 (18)	2 (25)	3 (12)	0.66
Hepatitis B or C	5 (7)	4 (10)	0 (0)	1 (4)	0.80
Pregnancy	4 (6)	2 (5)	0 (0)	2 (8)	0.78
Co-Infection *n* (%)					
Any	24 (33)	14 (36)	4 (50)	6 (24)	0.34
Bacteremia/fungemia[a]	3 (4)	3 (8)	0 (0)	0 (0)	0.45
Pneumonia	14 (19)	8 (22)	2 (33)	5 (21)	0.81
Non-bacterial pneumonia[b]	4 (6)	2 (5)	0 (0)	2 (8)	0.76
Other local infection	5 (7)	2 (5)	2 (33)	1 (4)	0.07
Other systemic infection	2 (3)	2 (5)	0 (0)	0 (0)	0.60
Immunocompromised *n* (%)					
Total[c]	35 (48)	21 (54)	3 (38)	11 (44)	0.63
HIV/AIDS	6 (8)	3 (8)	1 (13)	2 (8)	0.84
Transplant	19 (26)	12 (31)	0 (0)	7 (28)	0.23
Symptoms *n* (%)					
Fever	46 (58)	24 (62)	3 (38)	12 (48)	0.21
Cough	57 (72)	29 (74)	5 (63)	18 (72)	0.91
Diarrhea	13 (16)	6 (15)	0 (0)	7 (28)	0.51
Vomiting	16 (20)	5 (13)	2 (25)	8 (32)	0.37
White blood count *n* (%)					0.37
Low (< 4.5)	17 (23.6)	9 (29.0)	4 (50.0)	4 (21.1)	
Normal (4.5-11.0)	22 (30.6)	11 (35.5)	1 (12.5)	10 (52.6)	
High (>11.0)	19 (26.4)	11 (35.5)	3 (37.5)	5 (26.3)	
Hemoglobin ± SD	11.1± 2.3	10.8±2.1	11.3±3.9	11.5± 2.3	0.71
Platelet count ± SD	204±108	176±119	258±141	223±70	0.09
Elevated ALT[d] (>40) *n* (%)	13 (18.1)	10 (32.3)	0 (0.0)	3 (15.8)	0.11
Elevated AST[e] (>40) *n* (%)	14 (19.4)	11 (35.5)	0 (0.0)	3 (15.8)	0.07
Abnormal CXR *n* (%)	29 (40.3)	16 (41)	2 (25)	10 (40)	0.74
Treatment					
Any antibiotic	44 (60.3)	27 (77.1)	5 (100.0)	12 (54.6)	0.06
Oseltamivir *n* (%)	14 (19.2)	5 (16.7)	2 (40.0)	7 (31.8)	0.32

[a]Includes *Streptococcus bovis*, staphylococcal infection, *Candida*, *Cryptococcus*, *Pseudomonas*, and *E. coli*
[b]Non-bacterial pneumonia includes *Pneumocystis*, *Cryptococcus*, and CMV pneumonitis
[c]Patients with the following conditions were considered immunocompromised: HIV, post solid organ transplant, stem cell transplant, hematologic malignancy. Pregnancy, diabetes and renal failure were not considered immunocompromised states.
[d]ALT indicates the enzyme alanine aminotransferase
[e]AST indicates the enzyme aspartate aminotransferase

Table 2. Outcomes of patients with RV-A, RV-B and RV-C infection				
	RV-A (n=39)	**RV-B (n=8)**	**RV-C (n=25)**	**p-value**
Length of stay > 7 days[a]	14 (53.9)	1 (14.3)	2 (15.4)	0.03*
Death[b]	5 (12.8)	1 (12.5)	0 (0.0)	0.14
ICU admission	7 (26.9)	2 (33.3)	1 (7.7)	0.35
Death *or* ICU admission	8 (20.5)	3 (37.5)	1 (4.0)	0.04*

a. Length of stay did not follow a normal distribution and was therefore dichotomized into a categorical variable
b. Includes one patient, in RV-B group, who did not die in hospital but was discharged to hospice.

Table 3. Association of each risk factor with RV subtype and with outcome: assessment of potential confounders

Table 3a. p-value for the association of given risk factor with each outcome variable

Risk Factor	Outcome variables			
	Death	**ICU admission**	**Length of Stay**	**Composite outcome variable** (death or ICU admission)
Age	0.82	0.64	0.33	0.9
Sex	0.41	0.46	0.052	**0.03***
Malignancy	0.066	0.70	0.12	0.11
Pulmonary comorbidities	0.16	0.46	0.57	0.64
Co-Infection (any)	0.09	**0.02***	0.85	**<0.01**
Significant coinfection	0.62	0.06	0.34	**0.03***
Pneumonia	0.60	**0.04***	0.60	**0.01***
Immunocompromised	**0.01***	0.69	0.21	0.46

Table 3b. P-values for the association of each risk factor with RV type

Risk Factor	p-value for association of risk factor with RV subtype
Age	0.57
Sex	**0.05***
Malignancy	0.88
Pulmonary comorbidity	0.32
Co-Infection (any)	0.35
Significant coinfection	0.87
Pneumonia	0.66
Immunocompromised	0.59

Table 4. Adjusted odds ratio estimates from a logistic regression model of illness severity, controlling for patient sex

	OR estimate	95% CI	p-value
RV-A	4.4	0.5—39.8	0.18
RV-B	12.2	0.994—150	0.0506
RV-C	Reference	--	--
Female Sex	3.4	0.8—15.0	0.10

Table 5. Results of a logistic model of the effect of RV subtype on composite outcome of ICU admission or death, controlling for immunosuppression, Hepatitis B or C infection, malignancy, diabetes, hypertension, and significant coinfection[1]: with and without inclusion of sex in the model

	Sex omitted from model		*Sex included in model*	
	OR estimate	**p-value**	**OR estimate**	**p-value**
RV-A	7.9	0.09	6.0	0.15
RV-B	**20.7**	**0.037***	**18.1**	**0.054**
Immunosuppression	0.7	0.7	0.7	0.7
Hepatitis B or C	6.2	0.1	5.2	0.2
Malignancy	5.7	0.09	6.3	0.08
Diabetes	0.7	0.7	0.6	0.7
Hypertension	1.7	0.5	1.4	0.7
Co-infection	7.0	0.02*	6.3	0.03*
Female Sex	---	---	2.8	0.2

Table 6. Characteristics and demographics of patients with HRV infection: Excluded patients (those whose samples were unable to be amplified) vs. patients who were included in the study

	Study patients (included)	Excluded (non amplified)	p-value
Mean age (SD)	46.6 (16.7)	47.5	0.69
Sex			
Female *n* (%)	38 (52.8)	2 (20%)	0.052
Male *n* (%)	34 (47.2)	8 (80%)	
Comorbidities			
Hypertension (%)	32 (41%)	0 (0%)	0.01*
Diabetes (%)	9 (12.5)	4 (40%)	0.05*
Malignancy (%)	22 (30.6)	1 (20%)	0.13
Cardiac (%)	15 (20.8)	1 (10%)	0.68
Pulmonary (%)	22 (30.6)	6 (60%)	0.08
Renal (%)	12 (16.7)	0 (0.0%)	0.34
Hepatitis B or C (%)	5 (6.9)	0 (0.0%)	0.51
Pregnancy (%)	4 (5.6)	0 (0.0%)	0.59
Co-Infection (any)	24 (33%)	2 (20%)	0.49
Immunocompromised			
Total (%)	35 (49)	7 (70%)	0.2
HIV (%)	8 (10)	2 (20%)	0.25
Transplant (%)	10 (13)	4(40%)	0.45
Outcomes			
Death	6 (8%)	2 (20%)	0.22
ICU Admission	10 (21%)	3 (43%)	0.34
Death or ICU admission	12 (15%)	3 (30%)	0.36

Table 7. Power Calculation

Input Data	
Two-sided confidence interval (%)	95
Number of exposed[1]	47
Risk of disease among exposed[2] (%)	65
Number of non-exposed[3]	25
Risk of disease among non-exposed[2] (%)	31
Risk ratio detected	2
Power based on normal approximation:	*80%*

Results rounded to the nearest integer. Results from OpenEpi, Version 3, open source calculator—PowerCohort

1. "Exposed" = RV-A and RV-B. In this study, the number of exposed patients = RV-A (39) + RV-B (8) = 47. Based on Watanabe et. al, *Rhinovirus species and their clinical presentation among different risk groups of non-hospitalized patients (56)*

2. Risk of disease among exposed (65%) and non-exposed (31%) based on results for adult patients in *Rhinovirus species and their clinical presentation among different risk groups of non-hospitalized patients (56)*

3. "Non-exposed" = RV-C ; in this study *n* = 25

Figures and Figure Legends

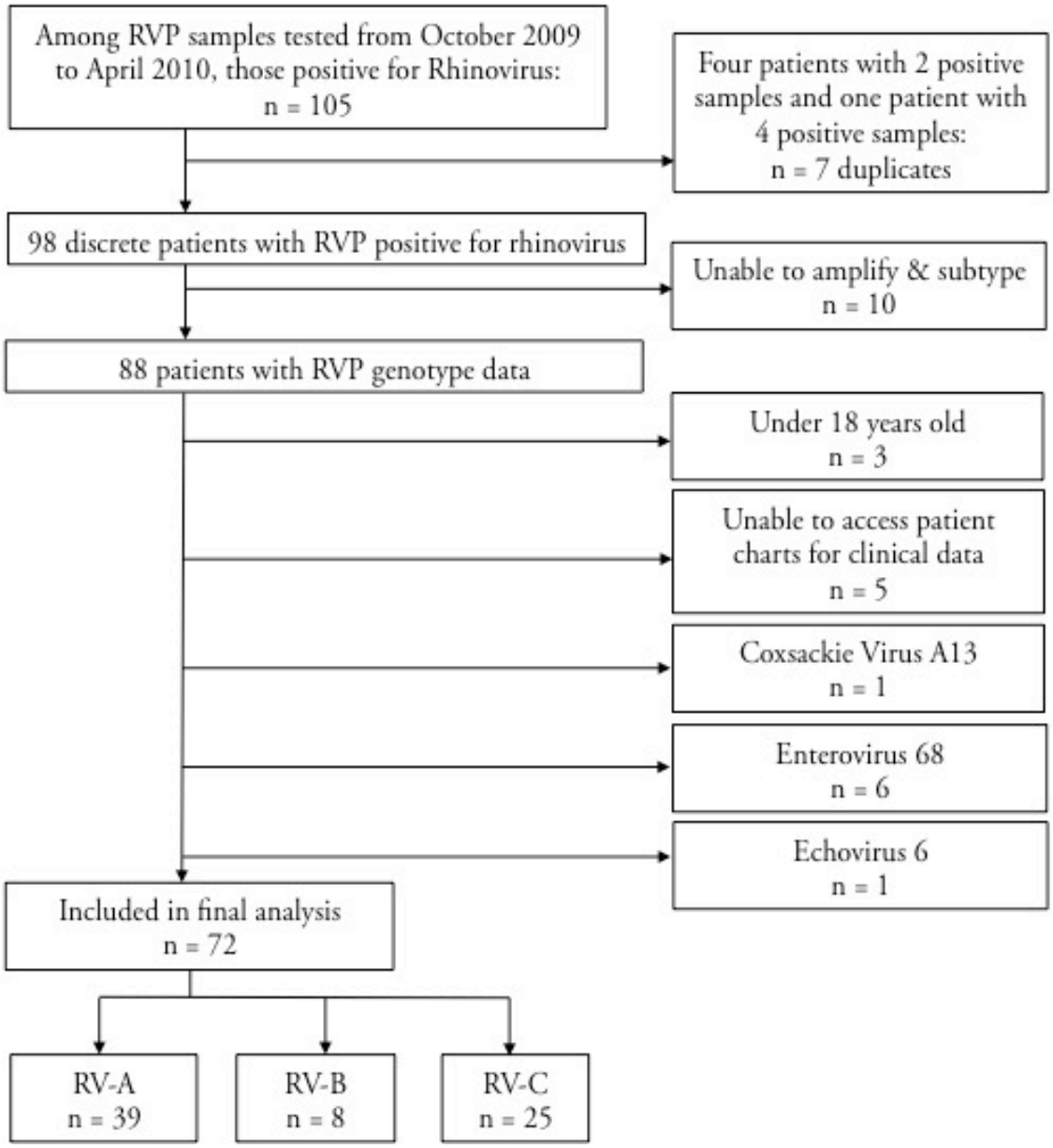

Figure 1. Algorithm of study inclusion beginning with all samples tested within the study timeframe. RVP = Respiratory Viral Panel, EV68= Enterovirus 68.

Appendix

Appendix A. Frequency of detection of RV-A, B and C in prior studies

	Number of isolates of each type of rhinovirus identified						
Study Title	**A**	**%**	**B**	**%**	**C**	**%**	**Total**
Distinguishing molecular features and clinical characteristics of a putative new rhinovirus species, human rhinovirus C (HRV C). (29)	34	64.2%	13	24.5%	6	**11.3%**	53
Rhinovirus species and their clinical presentation among different risk groups of non-hospitalized patients. (56)	74	60.7%	21	17.2%	27	**22.1%**	122
All known human rhinovirus species are present in sputum specimens of military recruits during respiratory infection. (72)	18	66.7%	5	18.5%	4	**14.8%**	27
Clinical severity and molecular typing of human rhinovirus C strains during a fall outbreak affecting hospitalized patients. (73)	45	52.9%	12	14.1%	28	**32.9%**	85
Phylogenetic Patterns of Human Respiratory Picornavirus Species, Including the Newly Identified Group C Rhinoviruses, during a 1-Year Surveillance of a Hospitalized Patient Population in Italy. (2)	110	56.4%	18	9.2%	67	**34.4%**	195
Role of Rhinovirus C Respiratory Infections in Sick and Healthy Children in Spain(46)	132	53.2%	28	11.2%	88	**35.4%**	248
Rhinovirus Genome Variation during Chronic Upper and Lower Respiratory Tract Infections (61)	71	55.9%	10	7.8%	47	**36.7%**	127
Clinical and Molecular Epidemiology of Human Rhinovirus C in Children and Adults in Hong Kong Reveals a Possible Distinct Human Rhinovirus C Subgroup (36)	111	50.5%	18	8.2%	91	**41.4%**	220
Human rhinovirus species and season of infection determine illness severity.(54)	257	49.5%	37	7.1%	225	**43.4%**	519
Evidence of recombination and genetic diversity in human rhinoviruses in children with acute respiratory infection (59)	27	40.9%	5	7.6%	34	**51.5%**	66
Human rhinovirus infection in young African children with acute wheezing. (44)	26	36.6%	8	11.3%	37	**52.1%**	71
Human rhinovirus species C infection in young children with acute wheeze is associated with increased acute respiratory hospital admissions. (52)	35	29.2%	3	2.5%	81	**67.5%**	120
Clinical features and complete genome characterization of a distinct human rhinovirus (HRV) genetic cluster, probably representing a previously undetected HRV species, HRV-C, associated with acute respiratory illness in children.(27)	5	19.2%	0	0.0%	21	**80.8%**	26
Genetics, recombination and clinical features of human rhinovirus species C (HRV-C) infections; interactions of HRV-C with other respiratory viruses.(74)	15	9.3%	2	1.2%	144	**88.9%**	162

Appendix B.
Review of the Literature: Prior Studies of the Relationship between RV species and Clinical Findings

Title, First Author	Population	Findings	Most severe
No difference			
Clinical features and complete genome characterization of a distinct human rhinovirus (HRV) genetic cluster, probably representing a previously undetected HRV species, HRV-C, associated with acute respiratory illness in children. Lau et al (27)	Children with acute respiratory tract infections.	No apparent difference between the clinical manifestations of HRV-A and HRV-C infections (but number of RV-A infections was small.)	No difference
Evidence of recombination and genetic diversity in human rhinoviruses in children with acute respiratory infection. Huang et al. (59)	Children with LRTI	RV-C did not have a greater clinical impact than RV-A or RV-B on respiratory compromise.	No difference
Rhinovirus genome variation during chronic upper and lower respiratory tract infections. Tapparel et al. (61)	RV + lung transplant recipients and hospital patients (children and adults)	No correlation between RV species and their ability to invade the lower respiratory tract or lead to protracted infection	No correlation
Human rhinovirus species associated with hospitalizations for acute respiratory illness in young US children. Iwane et al (60)	Children with ARI hospitalizations vs. control children	Clinical presentations similar among RV species.	No difference
Rhinoviruses Are a Major Cause of Wheezing and Hospitalization in Children Less Than 2 Years of Age. Piotrowska et al (43)	Children < 2 years old (some symptomatic, some asymptomatic)	No particular HRV strains are more likely to cause wheezing and asthma exacerbation than others. Likewise, it does not seem that certain isolates are more likely to cause severe disease and hospitalization than others.	No difference
RV-B most severe			
Host and viral factors associated with severity of human rhinovirus-associated infant respiratory tract illness. Miller et al. (58)	Infants aged less than 12 months were enrolled at the time of a clinical visit (hospitalization term, non–low birth weight, otherwise healthy infants	Infants with HRV-B infection were more likely to require supplemental oxygen and have a longer duration of hospitalization compared with infants with HRVA or HRVC. Infants with HRVB infection tended to have higher bronchiolitis severity scores, although the number of HRVB strains was small.	B (infants – oxygen, length of stay, severity)
Role of Rhinovirus C Respiratory Infections in Sick and Healthy Children in Spain. Calvo et al. (46)	Pediatric patients with respiratory tract infections	RV-C not significantly different from A. RV-B associated with more frequent infiltrate on CXR, fever, diagnosis of pneumonia	B (infiltrate, fever)

		and antibiotic treatment.	
RV-C most severe			
Human rhinovirus C associated with wheezing in hospitalised children in the Middle East. Miller et al. (49)	Hospitalized children	Compared with HRVA-infected children, children with HRVC were more likely to require supplemental oxygen (63% vs. 42%, p= 0.007) and, when co-infections were excluded, were more likely to have wheezing (100% vs. 82%, p= 0.016).	C (oxygen requirement; wheezing)
Clinical and Molecular Epidemiology of Human Rhinovirus C in Children and Adults in Hong Kong Reveals a Possible Distinct Human Rhinovirus C Subgroup. Lau et al- (36)	Hospitalized children and adults	(62%) of the 13 adults with HRV-C infection had pneumonia, compared with 6 (27%) of the 22 adults with HRV-A infection. Wheezing episodes were also more common among individuals with HRV-C (37%) and HRV-A (20%) infection than among those with HRV-B (0%).	C (adults – pneumonia)
Detection of human rhinovirus C viral genome in blood among children with severe respiratory infections in the Philippines. Fuji et al (51)	Hospitalized children with severe respiratory infections	HRVC may have a different pathogenicity and can more commonly cause viremia than HRVA and HRVB.	C (Children – viremia)
Human rhinovirus species C infection in young children with acute wheeze is associated with increased acute respiratory hospital admissions. Cox et al.(52)	Children less than 5 years of age, presenting to hospital with an acute wheezing episode	HRV-C–related wheezing illnesses are associated with an increased risk of prior and subsequent hospital respiratory admissions.	C (wheezing)
Patient characteristics and severity of human rhinovirus infections in children. Lauinger et al (53)	Children <16 years with episodes of respiratory tract infections	Monoinfection with HRV-C, as compared with other HRV species, was associated with more severe disease in young children <3 years	C (children < 3 years)
Clinical spectrum of human rhinovirus infections in hospitalized Hong Kong children. Mak et al (50)	Children with asthma exacerbations; inpatient controls	More subjects with RV-C needed oxygen (p=0.04)	C (children - oxygen)
Association between human rhinovirus C and severity of acute asthma in children. Bizzintino et al.(20)	Children with acute asthma	Children with RV-C had higher asthma severity scores (p=0.016)	C
Human rhinovirus C: Age, season, and lower respiratory illness over the past 3 decades. Linder et al (39)	Children < 5 years	HRV-C was significantly more common among children with LRI (60%; relative risk C vs A 5 2.152 [1.17-3.97]; P 5.014; Fig 4).	C (children - lower respiratory infection)
RV A and C			

Novel human rhinoviruses and exacerbation of asthma in children. Khetsuriani et al.(45)	Children with asthma ≥ 2 years of age	RV-A, RV-C associated with asthma exacerbations; patients with RV-C had lower FEV1.	A, C (asthma) C (lower FEV1)
Human rhinovirus C infections mirror those of human rhinovirus A in children with community-acquired pneumonia. Xiang et al (55)	Children with a diagnosis of community acquired pneumonia (CAP)	The severity of clinical manifestations for HRV-C is comparable to that for HRV-A in children with CAP	A, C (Children)
Human rhinovirus species and season of infection determine illness severity. Lee et al.(54)	Majority healthy infants, also infants with mild and moderate respiratory illness	HRV species A and C were each about seven times more likely than HRV-B to be associated with moderate to severe illness.	A, C (infants)
RV A and B			
Prevalence and clinical characterization of a newly identified human rhinovirus C species in children with acute respiratory tract infections. Jin et al. (42)	Children younger than 14 with RTIs	The number of patients requiring hospitalization was lower in the HRV-C monoinfection group than in the HRV-A or HRV-B monoinfection group (P = 0.028); however, the durations of hospitalization were not significantly different.	A, B (more hospital-izations)
Rhinovirus species and their clinical presentation among different risk groups of non-hospitalized patients. Watanabe et al.(56)	Non-hospitalized children and adults	HRV species A and B caused ILI among adult patients, whereas HRV-C did not.	A, B (adults - ILI)
Human rhinoviruses in Chinese adults with acute respiratory tract infection. Xiang et al (57)	Adult patients with acute respiratory tract infection	HRV-A infected patients had a higher percentage of upper respiratory symptoms than patients infected by the two other HRV species. Systemic symptoms such as chilliness and myalgia were more frequent in people infected by HRV-B.	A, B (adults – more symptomatic)