Conference Scene
Future Virology

# Current research on respiratory viral infections: XIII International Symposium on Respiratory Viral Infections: part 2

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## XIII International Symposium on Respiratory Viral Infections

Rome, Italy, 13–16 March 2011

The XIII International Symposium on Respiratory Viral Infections was convened by The Macrae Group LLC (NY, USA) in Rome, Italy, on the 13–16 March 2011. This annual symposium provides a forum for public health specialists, vaccinologists, clinicians, virologists and pharmacologists to discuss recent advances in respiratory virus research in an interdisciplinary fashion. Here, we highlight presentations at this conference with a special focus focus on respiratory infections other than influenza.

In part one of this Conference Scene article, we discussed presentations related to influenza from the XIII International Symposium on Respiratory Viral Infections, held in Rome, Italy on the 13–16 of March 2011. Part two highlights selected presentations on other viruses causing respiratory illness with a focus on their surveillance, burden of disease, and pathogenesis.

### Surveillance at the animal–human interface

The importance of surveillance at the animal–human interface is well-recognized for influenza, and increasingly appreciated for other viruses. Linfa Wang (Livestock Industries, Australian Animal Health Laboratory Victoria) discussed the value of bat surveillance to monitor for the emergence of novel epidemic diseases. Approximately 20% of all mammalian species are bats, and they are second only to rodents in terms of species diversity. Bats are important sources of zoonotic infections due to their widespread distribution, ability to fly, longevity and tolerance to viral infections. The significance of the bat population for surveillance efforts is highlighted by the emergence of several RNA virus infections with high mortality from bats, (e.g., Hendra virus [1994], Nipah virus [1998] and Meleka virus [2006]). During Nipah virus outbreaks in Malaysia human mortality rates were in the region of 40–50%, while in outbreaks in Bangladesh even higher mortality was noted.

Extensive sampling from wild bat populations has shown that they frequently harbor persistent viral infections that are lethal in nonbat populations. The reasons for this are still unclear, but Wang hypothesized that particular bat species, like the *Chiroptera*, have developed tolerance or resistance to a large number of viral diseases, with the innate immune system playing a role in allowing these persistent infections. In a surveillance study performed by Wang's team, urine collected from a local bat colony identified large, albeit transient populations of DNA and RNA viruses. In order to understand how and why these viruses are maintained within a bat population, a number of bat cell lines have been established as part of ongoing work to investigate the peculiarities of the bat immune system.

The increased number of zoonotic infections originating from bats seems likely to be due to environmental changes, human population growth and urbanization, which have led to increased contact between bats and humans. Wang presented data showing the spread of one bat species, *Pteropus alecto,* down the East coast of Australia since 1920 and indicated that the first transmission of Hendra virus into horses, which occurred in Brisbane, was only possible

Keywords
■ burden of disease ■ LMIC ■ respiratory viruses ■ rhinovirus ■ RSV ■ surveillance

because of the spread of this particular bat species into the area. In 2011, horse infections with Hendra virus have occurred as far south as Victoria and are ongoing in New South Wales and Queensland. There are increasing concerns about altered host range, as incidents of canine seroconversion have also been reported [101]. Wang concluded that surveillance of the animal–human interface, and bat populations in particular, is crucial. Combined with a better understanding of bat-virus interactions, it will lead to better detection and understanding of emerging novel threats.

## Burden of disease caused by respiratory viruses

Significant differences exist in the causes of death among children aged under 5 years in high income countries and low/middle income countries (LMIC). In Africa, 4.40 million deaths are reported annually, of which 21% are caused by pneumonia, whereas in Europe pneumonia accounts for 12% of the 0.26 million deaths in young children [1]. In order to prioritize strategies like immunization and antimicrobial therapy, an understanding of the causative infectious agents involved is crucial. Cristoph Steffen (Agence de Medecine Preventive, Ferney-Voltaire, France) presented a review of the burden of pediatric pneumonia and acute lower respiratory infection (ALRI) across LMICs, based on 28 community-based longitudinal studies in children aged <5 years. The 15 countries with the greatest absolute number of pneumonia deaths and pediatric pneumonia cases account for approximately 2 million ALRI deaths per year and nearly 116 million pneumonia cases [1]. Most fatal cases were associated with *Streptococcus pneumoniae*, followed in descending order by *Haemophilus influenzae* type B, respiratory syncytial virus (RSV) and influenza infections. The highest mortality rate was observed in Africa, but substantial variation existed in mortality rates of different geographical regions. Steffen cautioned that that the modeled estimates were not robust, as they are based on limited data from too few countries, and highlighted the crucial need for more data across LMICs.

This issue is being addressed to some extent, and four investigators presented results of surveillance studies in LMICs. Xiu-Feng Wan (Institut Pasteur, Shanghai, China) discussed an ongoing hospital-based study from Shanghai designed to identify patterns of pathogen coinfections in mild and severe acute respiratory infections (MARI and SARI). As Shanghai has 4 million short-term migrants in a total population of over 19 million, it has the potential to be a center for mixing and transmission of respiratory diseases. During an initial 15 month period of surveillance, nasopharyngeal swabs collected from 1081 children with either MARI (1035) or SARI (46) were tested for viral and bacterial infections by multiplex and specific PCR. The most frequently detected viruses in SARI cases were adenovirus (28%) and human rhinovirus (HRV; 28%), while in MARI cases influenza A virus (37%) was most frequently detected. In 6.5% of MARI and 10.9% of SARI cases, multiple respiratory viruses were found. In both MARI and SARI, the most frequently detected bacterial species were *S. pneumoniae* and *Mycoplasma pneumoniae.* Coinfections with these bacteria and viruses were more common in SARI (15.2% and 6.5%, respectively) compared with MARI cases (6.6 and 0.6%, respectively), supporting an association of bacterial coinfection with greater disease severity. However, Wan noted that it is difficult to establish whether viral or bacterial infection is the primary cause of disease.

Nusrat Homaira (International Centre for Diarrhoeal Disease Research, Bangladesh) described a year-long, prospective, community-based cohort of 415 children, aged 0–2 years, in an urban slum setting, who were monitored by twice weekly visits. Nasal washes were taken from symptomatic children, and tested by rRT-PCR for different viruses. The clinical diagnosis of acute respiratory illness required one major sign (fever >38°C, rapid/labored/noisy breathing, or danger signs lethargy/cyanosis/convulsion) or two minor signs (e.g., cough, rhinorrhoea, sore throat, chills and headache). Pneumonia was diagnosed clinically based on age-specific respiratory rates. The incidences of acute respiratory illness and pneumonia were 0.9 and 0.6 cases per child per year, respectively. Pneumonia was most commonly associated with RSV (24%), especially in children under 2 years of age, and with HRV (21%), but viral coinfections were common in both syndromes. Homaira concluded that in a poor urban setting in Bangladesh, childhood pneumonia is common, with rates comparable to those seen in poor rural settings in African countries.

James Nokes (KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya) also highlighted the importance of RSV-associated pneumonia, in the rural coastal district of Kenya. In a birth cohort, followed from 2002 to 2005, around 1% of infants were hospitalized in first year of life with severe RSV pneumonia. However, only 20% of children with severe RSV were admitted to the hospital, the rest being cared for in the

community [2]. Hospital and community-based surveillance between 2007 and 2010 confirmed the role of RSV as a major viral cause of ALRI and pneumonia and also showed that RSV infection was highly seasonal, with major peaks in the dry season (December to March). Among children aged up to 12 years with severe or very severe pneumonia, RSV alone was detected in 20% of cases, 5% had RSV coinfection with another virus and 36% had other respiratory viral infections (RVIs) detected, of which HRV was most common. The remaining 39% had no detectable viral infection. The majority of cases were in infants aged 0–2 months, which presents a real challenge for developing effective vaccines and raises the possibility of alternative immunization strategies like maternal vaccination or targeting siblings or schools to reduce community transmission. Currently, Nokes is conducting a household-based study, designed to help understand rates of infection, transmission patterns within households and interactions between viral and bacterial infections.

Also working in Kenya, Janet Majanja (Kenya Medical Research Institute, Nairobi) presented results from disease surveillance conducted since 2006 in close collaboration with the US Department of Defense Global Emerging Infections Surveillance and Response System (DoD-GEIS). Surveillance focuses on RVIs and the noninfluenza burden of disease at eight surveillance sites throughout the country. Specimens from patients with influenza-like illness are tested for influenza virus by RT-PCR and other RVI by conventional virus isolation methods in a central Nairobi laboratory. From a total of 12,959 samples, RVIs were detected in 2592 (20%), with influenza identified in 959 (37%) of the RVI-positive samples. Other common RVIs were parainfluenza (predominantly types 1 and 3) in 24%, adenovirus in 17%, and RSV in 12% of positive samples. Majanja concluded that the results underline the importance of noninfluenza RVIs causing influenza-like illness in Kenya and are probably an underestimation of the burden because of the lower sensitivity of conventional virus isolation methods compared with the RT-PCR used for influenza virus detection. Further longitudinal studies in a wider range of countries are needed to generate a clearer picture of the burden of disease from various RVIs.

Even in high income countries, there remain clinical situations where data is insufficient regarding the RVI-associated burden of disease. Terho Heikkinen (Turku University Hospital, Finland) described the importance of viruses in acute otitis media (AOM). Over 80% of AOM cases have symptoms typical of a RVI (rhinitis and cough), and the highest incidence of AOM is 3–4 days after onset of symptoms [3]. Bacteria can be cultured from the middle ear fluid in around 70% of cases, such that AOM is generally regarded as a bacterial complication of a RVI. However, the clinical response to antibiotics is often poor. Mechanistically, Heikkinen hypothesized that Eustachian tube dysfunction (including impairment of pressure equilibration and decreased secretion drainage) due to RVI-associated inflammation allows for microbial invasion, further inflammation and ultimately AOM. Using molecular diagnostics, he and others have shown the presence of RVIs (often in conjunction with bacterial infection) in over 65% of cases of AOM [4–6]. Of these infections, over 60% are accounted for by HRV, nontypeable picornoviruses and enterovirus, indicating a potential value for specific antiviral therapy directed against HRV and related viruses [6].

In immunocompromised hosts, RVIs are important causes of morbidity, hospitalization and sometimes death. Michael Boeckh (Fred Hutchinson Cancer Research Center, WA, USA) discussed his group's efforts to characterize RVIs in this group of patients, particularly in hematopoietic stem-cell transplant (HSCT) recipients. Their results indicating that HRV and coronaviruses are the most commonly detected RVIs during the first year after HSCT, with about 30% developing HRV infection and 16% developing coronavirus infection. Clinically HRV infection usually presents as an upper respiratory tract infection, but occasionally progresses to lower respiratory tract infection (LRI) and in approximately 1% of HSCT recipients to a fatal outcome. A correlation appeared to exist between the upper respiratory tract viral load and disease severity. Other RVIs, such as bocavirus and Wu/Ki polyomaviruses, were detected in less than 5% of patients and did not appear to cause LRI. Understanding contributors to disease progression and identifying biomarkers are important elements in predicting and preventing disease progression. In an analysis of 150 RSV cases, Boeckh found progression to LRI in 25% of cases. Among possible contributory factors, only lymphopenia correlated significantly with increased risk of progression to LRI. Both respiratory tract RSV load and viral RNA detection in blood appeared to be associated with disease severity [7]. Boeckh concluded that further studies are needed to define the pathogenesis of LRI due to RVIs in HSCT patients.

## Pathogenesis of respiratory viruses

### Respiratory syncytial virus

In the USA, RSV infects up to 60% of the entire birth cohort within the first year of life and is responsible for hospitalization of nearly 3% of them [8,9]. John DeVincenzo (University of Tennessee, USA) gave two presentations related to his work on RSV pathogenesis and establishing immune correlates of protection. RSV infects the human respiratory epithelium during acute disease, but may also cause infection of the lungs in infants and other risk groups. In the pathogenesis of pediatric RSV illness, the virus does not appear to induce prominent $CD4^+$ and $CD8^+$ T-cell responses, and in fatal cases of RSV infection, histopathologic studies show a notable lack of immune effector cells and little granzyme release. There is, however, a strikingly high level of apoptosis and lung damage. Unfortunately, determining the causes of lung damage is challenging in high-income settings because prolonged mechanical ventilation can itself cause lung injury. In a past study undertaken in Santiago, Chile when mechanical ventilation was less common, autopsy samples from RSV cases dying 5–6 days after illness onset were compared with fatal influenza cases [10]. The alveoli of RSV infected patients were filled with viral particles, something not seen in influenza or uninfected lung tissue. Such evidence for high RSV replication levels suggests a role for antiviral therapy.

Given the sometimes rapid progression to severe disease, a central role presumably exists for innate immune responses to RSV. A number of candidate gene analyses of innate immune system-related genes have been conducted to identify SNPs linked to disease progression. A study published by DeVincenzo showed a consistent correlation between particular SNPs in the gene for Surfactant protein A and RSV illness severity in children aged ≤24 months [11]. Surfactant protein A is a collectin with carbohydrate recognition domains in its head and a stalk, and these domains help coordinate the immune response and may therefore be important in resistance to RSV. However, DeVincenzo concluded that wider whole-genome approaches are needed to look for other important host genetic factors.

He also described the use of the RSV human challenge model to study the correlations of pre-existing serum and mucosal antibody levels with RSV susceptibility and disease severity. Adults to be enrolled into the study are screened for serum levels of RSV microneutralizing antibody to the challenge strain, and only those in the bottom 25% of the population are enrolled. Volunteers are inoculated intranasally with a GMP-manufactured, low passage, wild-type RSV (Memphis 37) [12]. In adults, nasal viral load started to increase 2–3 days after inoculation and illness severity is closely linked to nasal lavage virus titers. By day 6 post-inoculation the frequency of culture positivity drops and clinical symptoms begin to resolve. Most subjects continue to have detectable nasal viral RNA for some days, but it is unclear whether this represents infectious virus or clearance of debris containing viral RNA. However, the detection of positive-strand RNA suggests that low-level replication is still ongoing. In 35 subjects studied to date, the level of RSV-specific IgA in the nasal wash of subjects who were successfully infected was significantly lower than in those who were resistant to infection. Higher levels of RSV-specific IgA also correlated to lower levels of viral replication and trended towards less-severe disease. However, once patients developed RSV infection, higher pre-existing levels of IgA or higher microneutralisation titers did not reduce illness severity.

The ultimate aim of the work is to understand immunity and define correlates of protection as a critical step for the future development of vaccines. Despite the discovery of RSV more than 50 years ago, no vaccine or highly effective antiviral treatment currently exists. Monthly injections of an RSV monoclonal antibody palivizumab can reduce RSV hospitalizations by 50–60% in at-risk infant populations, but is only applied to <3% of the birth cohort because of its high cost. For this reason, WHO has listed an RSV vaccine as one of the highest priority vaccine needs.

### Human rhinovirus

HRV infections usually cause mild upper respiratory illnesses but are also major drivers of asthma and chronic obstructive pulmonary disease (COPD) exacerbations. Asthma exacerbation is associated with RVI in up to 95% of cases in preschool children, 85% of cases in school-aged children and 80% of cases in adults. Viral and bacterial infections also play key roles in COPD exacerbation, where 24% of cases are associated with RVI, 30% with bacterial infection and 25% with a combination of the two [13]. Sebastian Johnston (National Heart & Lung Institute, Imperial College London, UK) provided a summary comparing usual immune responses to HRV infection to those observed in a variety of asthma or COPD models. In studies of experimentally induced HRV infections, atopic asthmatics (naive for steroids) had increased chest symptoms and falls in peak expiratory

flow compared with nonatopic healthy subjects. Bronchoalveolar lavage (BAL) samples from asthmatics showed deficient IFN-λ responses and also reduced induction of the antiviral cytokine IL-15, which is important in NK and memory CD8 T-cell responses. In addition, *ex vivo* HRV infection of primary bronchial epithelial cells obtained from healthy and asthmatic subjects showed marked induction of IFN-β in healthy individuals but not in asthmatics. IFN-λ induction is also deficient in HRV-infected primary epithelial cells from asthmatics. The mechanism of deficient induction of IFNs in asthmatics in response to HRV is not known. While various proteins like TLR3, 7, 8, RIG-I, MDA5 and TRIF are required, they do not appear to be deficient in BAL cells from infected asthmatics. In a related talk, Vera Gielen (Imperial College London) discussed studies examining modulation of the IFN response induced by HRV, with a focus on suppressors of cytokine signaling SOCS1 and SOCS3 in human bronchial epithelial cells. SOCS1 and SOCS3 are from an eight member protein family, all of which function by inhibiting cytokine–cytokine receptor-mediated signaling. SOCS1 suppresses IFN-α/β and IFNγ-mediated signaling and is upregulated upon HRV infection. In addition, increased SOCS1 mRNA levels in human bronchial epithelial cells from asthmatic children correlate with reduced IFNβ levels. Gielen concluded that further work is needed to uncover the exact roles of SOCS in reducing induction of IFNs in asthmatics.

Nathan Bartlett (NHL Institute, Imperial College London) presented results of a study to investigate HRV-induced allergic inflammation in a murine model of HRV-induced asthma exacerbation [14]. In this model, HRV infection induces the production of Th2 cell-recruiting chemokines like CCL17 and CCL22, and Bartlett hypothesized that these chemokines then drive Th2-allergic inflammation and exacerbation of asthma. HRV infection alone does not seem to drive CCL17 and CCL22 production, but HRV infection in conjunction with an ovalbumin challenge leads to marked increases. In addition, allergen and HRV together lead to increased numbers of $CCR4^+$ cells in BAL samples, as well as increased IL5 and IL13. Bartlett concluded that although the mechanism of activation is yet to be demonstrated, the promotors of CCL17 and CCL22 do contain NFκB and STAT5/6 binding sites; HRV infection induces activation of both NFκB and STAT5/6. In the future this murine model may assist in drug development.

A study using a human challenge model investigating HRV-induced exacerbation of COPD was presented by Joseph Footitt (Imperial College). For inclusion in the study COPD patients had to be at the milder end of the disease spectrum for safety reasons (Gold stage 2) and were monitored closely for 14 days after intranasal inoculation. Following HRV infection, LRT symptoms were more severe and more prolonged in COPD subjects compared with healthy controls. In addition, neutrophilic and lymphocytic airway inflammation was increased in COPD subjects, as were nasal lavage and sputum viral loads which correlated with disease severity. Comparable to asthmatic subjects, BAL cells of COPD patients showed impaired IFN production. These findings indicate that viral replication is a driver for appearance of clinical symptoms, suggesting a potential benefit of antiviral interventions. Currently, one possibility under current investigation is inhaled IFN-β which, given daily for a period of 14 days, induces IFN-dependent antiviral genes in lung cells of asthmatic test subjects. Footitt concluded that this human model may also provide a platform for testing of other novel therapeutics.

**Acknowledgements**

*The authors would like to thank The Macrae Group LLC for organization of the XIII ISRVI and its sponsors: Viroclinics Biosciences (Rotterdam, The Netherlands), Armed Forces Health Surveillance Center (Silver Spring, MD, USA), Department of Defense Global Emerging Infections System (Silver Spring, MD, USA), Life Technologies (Carlsbad, CA, USA), GlaxoSmithKline (Philadelphia, PA, USA), F Hoffmann-La Roche Ltd. (Basel, Switzerland), Wellcome Trust (London, UK) and The Macrae Group LLC (New York, NY, USA).*

**Financial & competing interests disclosure**

*Since 2008 FG Hayden has served as an unpaid consultant to multiple companies involved in influenza antiviral and vaccine development. AS Monto reports receiving consultation fees from GlaxoSmithKline, Novartis, Roche and Biocryst and grant support from Sanofi-Pasteur. A Osterhaus is head of the Department of Virology of Erasmus MC Rotterdam and part-time CSO of Viroclinics-Biosciences BV, a spin-off CRO of Erasmus MC that collaborates with pharmaceutical companies.* ***The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.***

*No writing assistance was utilized in the production of this manuscript.*

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## Website


101. Confirmed Hendra virus case. Promed mail daily update on 13 October 2011 http://beta.promedmail.org/direct.php?id=20111013.3061