Kelly J. Henrickson
John V. Williams
David Spiro
Martha Nelson

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White Paper

Deficiency of Bioinformatic, Phylogenetic, and Evolutionary data For the majority of common community acquired respiratory viruses infecting humans. (genus paramyxoviridae).

The Paramyxoviridae family is a large rapidly growing group of viruses that cause significant human and animal disease (table 1). This virus family is one of the most costly in terms of disease burden and economic impact to our planet. Recently discovered paramyxoviruses (Hendra, Nipah, and human metapneumovirus [HMPV]) emphasize this point. A typical virus has a single negative strand of RNA in its genome and is surrounded by a lipid envelope of host cell origin. These medium size viruses (150-200 nm) are all similar in their structural, physiochemical, and biological characteristics except that Pneumo and Metapneumoviruses have narrower nucleocapsids.

Human parainfluenza virus (HPIV) 1,2,3, respiratory syncytial virus (RSV), and human metapneumovirus (HMPV) as a group cause significant upper and lower (LRI) respiratory infections in young children, the elderly, immunocompromised patients and those with chronic medical disease. Although these viruses pose a significant health and economic burden on adults there are more data on their burden in the pediatric population as described below.

Despite advancements in prevention and therapy, LRI continue to be a significant cause of morbidity and mortality in children world-wide (28-30, 31-39). The number of children < 5 years of age with LRI in the USA is estimated at greater than 5 million (19, 40). The annual incidence of pneumonia in children younger than 5 years of age is 34 to 40 cases per 1000 in Europe and North America (41). Pneumonia remains a major cause of morbidity and mortality with over 220,000 children less than 18 years of age hospitalized each year in the United States (28-30). Estimates of national disease burden of respiratory viruses, using lab confirmed cases at the Children's Hospital of Wisconsin, were previously published by our group (4). An estimated 300,000 children are hospitalized each year in the United States with a laboratory diagnosis of viral LRI, and an additional 500,000 children are hospitalized with a clinical diagnosis of viral LRI, at a direct cost estimated at nearly $1 billion per year (4). RSV has been estimated to result in more than 90000 -112000 hospitalizations annually in children < 5 years of age (4, 19, 40, 42-43). HPIV has been estimated to result in >65000 hospitalizations among children every year in the United States (4, 8, 20-22, 40, 45). In comparison Influenza infections are associated with an estimated 25000 -28000 hospitalizations among young children < 5 years of age annually (4, 44, 47-49). HMPV causes an estimated 27,000 pediatric hospitalizations each year in the US (Williams et al, unpublished data). The national cost of viral infections in children was estimated to be $190 million/year for PIV (45), $300-500 million dollars/year for RSV (50, 51) compared to ~ $55 million for influenza (52, 53).

The direct medical costs incurred for hospitalized children with viral LRI have been estimated to be 2.4 billion (3). In addition to the well described disease burden posed by influenza, RSV and PIV associated hospitalizations, other viruses that have been more recently described to have been associated with significant lower respiratory tract illness resulting in hospitalizations include human metapneumovirus (54-57. Precise national economic burden from this newly discovered respiratory pathogen has yet not been published. To reduce this enormous burden imposed by paramyxovirus LRI in children, it is essential to accurately detect these viruses and to understand their evolution and natural history. This will require adequate whole virus genome data for a significant number of these viruses (**good paramyxovirus bioinformatics**). This will include sequencing virus isolates collected over time from diverse geographic locations. This will allow us to define the current true epidemiology of these infections so as to identify virus specific targets of pathogenicity or allow discovery of markers (correlates) for host specific immunity. This knowledge will lead to future research and development of new vaccines, diagnostics and therapeutic agents.

Gaps in current knowledge of epidemiology of pneumonia in children. Despite a multitude of epidemiologic investigations over decades, the precise epidemiology of pneumonia in children remains poorly defined. Studies using an expanded spectrum of both conventional diagnostic methods and emerging molecular assays have reported up to 50 -90% of pneumonias in preschool children to be viral with a small percentage of that mixed viral/bacterial. These rates then decrease to approximately 50% of young school children and eventually to 10-15% of adolescent and adult pneumonias being caused by viruses (19, 40).

The viruses most commonly associated with LRI are influenza, RSV, HPIV-3, and HMPV with smaller contributions by HPIV-1, 2 and other community-acquired viruses, as well as newly discovered agents. The relative distribution of the presently known viruses to LRI in positive diagnostic samples is shown in **Fig 1.** (2). Ongoing studies to determine the epidemiology of viral pneumonia remain important to identify new respiratory pathogens, better understand the roles of less well characterized viral agents, and to gain appreciation of the shifting burden from well known agents. However, the lack of current molecular data on the genomes of common human paramyxoviruses contributes to decreased ability to fully understand the true burden and epidemiology of these viruses. In addition, it limits our ability to create rapid and accurate molecular diagnostic tests.

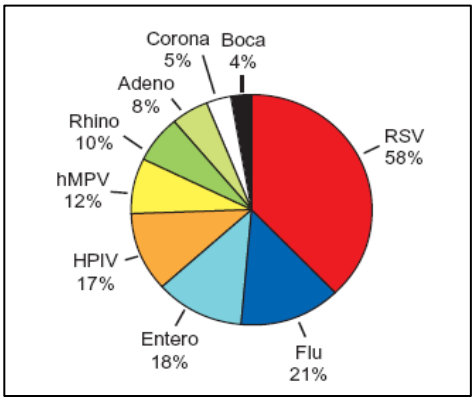

Fig 1. Laboratory-confirmed common viruses causing serious LRI in children/adults younger than 19 years of age (2).

RSV. Studies evaluating RSV and influenza associated LRI have revealed the shifting demographic and epidemiologic patterns of these viruses and their impact on hospitalization and mortality (67). Recognition of dramatic (more than 2 fold) increases in hospitalization rates from RSV between 1980 and 1996 (39,67) led to accelerated research efforts to develop measures to reduce this health care burden including development of active (experimental vaccines) and passive immunity measures (monoclonal antibodies) for RSV. A prospective population based study conducted by the NVSN (between October 1, 2000, and September 30, 2001) to determine the burden of acute respiratory illness (ARI) hospitalizations from RSV, influenza and PIV, demonstrated RSV to be the leading pathogen associated with ARI hospitalizations. The

13, 3, and 0.4 RSV-associated hospitalizations per 1000 children younger than 1 year, 1 year, and 2 to 5 years, respectively were within the same order of magnitude of previously estimates rates of 15 and 40 per 1000 children younger than 1 year, 2 to 10 per 1000 children 1 year of age, and 1 to 2 per 1000 children between 2 and 5 years of age both demonstrating the huge burden this one paramyxovirus causes to society.

Palivizumab, the only approved monoclonal antibody for prophylaxis of RSV has been shown to reduce hospitalization rates (68, 69) in high-risk infants but remains ineffective for a small number of patients and does not inhibit replication of RSV in the upper respiratory tract. Exciting developments in the field of RSV research have included development of monoclonal variants (70, 71) and a live attenuated vaccine. Motavizumab, an ultra-potent, affinity-matured, humanized mAb derived from palivizumab is currently before the FDA. A significant milestone in viral research has included development of a bivalent live attenuated vaccine against RSV and PIV3, also now being studied in pediatric clinical trials (72).

However, the lack of genomic data on RSV is crippling our ability to understand where we are today (what is the genomic diversity on our planet of RSV?), how will the virus/host interaction change with vaccines or with new high affinity mAb therapeutics? How will the virus evolve under these new pressures within populations and within individuals?

Parainfluenza. These viruses are second only to RSV as a cause of hospitalizations for LRI in children (4, 20-22, 45, 73) A large scale population based study done by our group at CHW (45) to determine epidemiology and cost of PIV infections revealed that the national burden of LRI amounted to 250,000 emergency-room visits and approximately 70,000 hospitalizations due to HPIV-1 and HPIV-2, with a cost of $50 million for the former and $140 million for the latter. Subsequent studies demonstrated significant temporal and geographic variability in the incidence of HPIV-2. Using a highly sensitive multiplex PCR assay to determine epidemiology of respiratory viruses in hospitalized children (<18 years of age), 17% of all viruses identified were positive for PIV in a subsequent study conducted by our group (4). A study conducted by the NVSN (children enrolled between 2000 to 2004) demonstrated that PIV accounted for 6.8% of hospitalizations for ARI in children under 5 years of age, with the mean PIV hospitalization rates being 3.01, 1.73, 1.53, 0.39, and 1.02 per 1000 children per year for ages 0 to 5 months, 6 to 11 months, 12 to 23 months, 24 to 59 months, and 0 to 59 months. (74) Several PIV vaccine candidates including intranasally administered bovine PIV3 (bPIV3) vaccine and cold-adapted PIV3 vaccine have been evaluated in children for prevention of PIV3 infections, the most prevalent of the 4 types of PIV(75). The most recent approach, using bPIV3 vaccine as an attenuated backbone for insertion of human PIV3 hemagglutinin-neuraminidase (HN) and fusion (F) proteins and the RSV F protein, has just entered clinical trials. These advancements are expected to result in reduced burden from RSV and PIV3, two of the most common respiratory infections in childhood. However, paramyxoviruses mutate readily due to encoding an error-prone RNA polymerase. The effect of vaccine-induced immune pressure on genetic and antigenic variability of these viruses is knot know. Greater knowledge of natural genetic variability between geographic locations and over time will inform the development of broadly protective vaccines.

One of the most important human viruses discovered and studied in the last decade is HMPV (2001) (55, 57,76). HMPV has been well established as a significant respiratory pathogen in both children and the elderly. The NVSN described the epidemiology of this virus in 2004 using molecular tests although the study was performed using frozen banked specimens collected prior to the discovery of this virus (82). Several studies since then have emerged that continue to describe the epidemiology of this virus in children and adults (55,57, 83-84).

What is known about the genomics of common community acquired paramyxoviruses that cause respiratory tract disease (see Table 2)

	Whole genome	partial genomes
1. RSV (A,B)	**25**	**4320***
2. HPIV-3	**5**	**109**
3. HMPV (A,B)	**11**	**3469**
4. HPIV-1	**1**	**118**
5. HPIV-2	**5**	**267**
6. HPIV-4 (A,B)	**1**	**39****

* The vast majority of these sequences are from research clones that are mutated to create vaccine candidates and do not add to our knowledge of biodiversity or evolution.
**34 of these are to a portion of the P gene.

What is evident is that almost no work has been done over the last 25 years investigating whole genomic diversity, evolution or natural selection in this large group of human pathogens. We have established the enormous burden of disease caused by this family of viruses and the lack of data to truly understand these pathogens on a geographic and temporal basis.

Rapid high throughput whole genomic sequencing of influenza virus has been established through a partnership of many investigators and the NIAID led by Drs. David Spiro and Maria Giovanni. Their group has demonstrated the ability to receive samples from all over the world and of considerable age and recover complete genomic data. The MRVP led by Dr. Henrickson has contributed a large portion (2/3) of the genomic diversity data know for HPIV-1 and 3 (some of this currently unpublished) and the MRVP laboratory has been working on a rapid whole genomic sequencing protocol for RSV. Dr. Williams has contributed significant genomic diversity data on HMPV and led the field in understanding the evolution of this virus. We believe as a group we can establish a program for high throughput whole genomic sequencing of the important paramyxoviruses causing human disease. Secure the appropriate clinical samples and rapidly analyze and broadly disseminate this information throughout the general scientific and medical community. In addition, this service would be made widely available to all investigators interested in contributing to our knowledge of these viruses. We strongly urge the NIH to establish research funding to support this broad agenda.

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TABLE 1. TAXONOMIC RELATIONSHIPS OF HUMAN PARAINFLUENZA VIRUSES.

PARAMYXOVIRIDAE FAMILY

	HUMAN	ANIMAL
PARAMYXOVIRINAE (SUBFAMILY)		
RESPIROVIRUS (genus)	PIV-1 & 3	SENDAI (MOUSE PIV-1)
		BOVINE PIV-3
		SIMIAN PIV-10
RUBULAVIRUS (genus)	PIV-2 & 4A, 4B MUMPS	LA-PIEDAD-MICHOACAN-MEXICO (PORCINE) SIMIAN PIV 5 & 41
MORBILLIVIRUS (genus)	MEASLES	CANINE DISTEMPER RINDERPEST (BOVINE) PEST-DES-PETITS-RUMINANTS DOLPHIN DISTEMPER PORPOISE DISTEMPER PHOCINE DISTEMPER
HENIPAVIRUS (genus)	Hendra virus Nipah virus	
AVULAVIRUS (genus)		Newcastle disease virus (avian Parainfluenza virus 1) Avian paramyxoviruses 2-9 (Yucaipa (2), Kunitachi (5)
TPMV-LIKE VIRUSES (genus)		Tupaia (shrew)
PNEUMOVIRINAE (SUBFAMILY) PNEUMOVIRUS (genus)	RSV	BOVINE RSV PNEUMONIA VIRUS OF MICE
METAPNEUMOVIRUS (genus)	HMPV	AVIAN METAPNEUMOVIRUS

Table 2. Known subgenomic sequences for the human respiratory Paramyxoviridae.

Organism	Gene	Total						
HPIV1	L	19	HPIV2	F	38	HPIV3	D	9
	N	29		HN	73		F	14
	HN	64		L	19		HN	44
	F	11		M	8		L	28
	C	4		N	132		M	10
	C'	5		P	7		N	19
	M	7		Unk	23		P	12
	P	8		Total	300		Unk	21
	Y1	4					Total	157
	Unk	10						
	Total	161						
HPIV4	F	6	HMPV	F	1643	RSV	F	280
	HN	4		G	279		G	3277
	L	2		L	282		L	84
	M	5		M	83		M	36
	N	5		M2-1	27		M2-1	30
	P	40		M2-2	23		M2-2	25
	Unk	0		N	574		N	380
	Total	62		P	730		NS1	111
				SH	23		NS2	86
				Unk	189		P	45
				Total	3853		SH	221
							Unk	226
							Total	4801