and B influenza viruses: Basis for the WHO recommendation on the composition of influenza vaccines for use in the 2009–2010 Northern Hemisphere season. Writing Committee of the World Health Organization Consultation on Northern Hemisphere Influenza Vaccine Composition for 2009–2010. Vaccine 2010; 28:1156–1167.

FDA/NIH/WHO. Public workshop on immune correlates of protection against influenza A viruses in support of pandemic vaccine development. Report of a meeting held in Bethesda, Maryland, United States, December 10–11, 2007. Vaccine 2008; 26:4299–4303.

Fouchier RAM, Smith DJ. Use of antigenic cartography in vaccine seed strain selection. Avian Dis 2010; 54:220–223.

Russell CA *et al.* Influenza vaccine strain selection and recent studies on the global migration of seasonal influenza viruses. Vaccine 2008; 26S:D31–D34.

Smith DJ. Applications of bioinformatics and computational biology to influenza surveillance and vaccine strain selection. Vaccine 2003; 21:1758–1761.

WHO. A description of the process of seasonal and H5N1 influenza vaccine virus selection and development. Draft version. 2007. See: http://apps.who.int/gb/pip/pdf_files/Fluvaccvirusselection.pdf.

WHO. Recommended composition of influenza virus vaccines for use in the 2010 influenza season (southern hemisphere winter. Wkly Epidemiol Rec 2009; 84:421–436.

WHO. Recommended viruses for influenza vaccines for use in the 2010–2011 northern hemisphere influenza season. Wkly Epidemiol Rec 2010; 85:81–92.

WHO. Antigenic and genetic characteristics of influenza A(H5N1) and influenza A(H9N2) viruses and candidate vaccine viruses developed for potential use in human vaccines – February 2010. Wkly Epidemiol Rec 2010; 85:100–108.

Improving influenza surveillance and representative virus sharing

Moore M *et al.* Strategies to improve global influenza surveillance: a decision tool for policymakers. BMC Public Health 2008; 8:186.

Schoub BD, McAnerney JM, Besselaar TG. Regional perspectives on influenza surveillance in Africa. Vaccine 2002; 20:S45–S46.

WHO. Global influenza surveillance network: laboratory surveillance and response to pandemic H1N1. Wkly Epidemiol Rec 2009; 84:361–372.

Improving the process of vaccine virus selection

Abdiche Y *et al.* Determining kinetics and affinities of protein interactions using a parallel real-time label-free biosensor, the Octet. Anal Biochem 2008; 377:209–217.

Bose ME *et al.* Rapid semiautomated subtyping of influenza virus species during the 2009 swine origin influenza A H1N1 virus epidemic in Milwaukee, Wisconsin. J Clin Microbiol 2009; 47:2779–2786.

Hassantoufighia A *et al.* A practical influenza neutralization assay to simultaneously quantify hemagglutinin and neuraminidase-inhibiting antibody responses. Vaccine 2010; 28:790–797.

Ince J, McNally A. Development of rapid, automated diagnostics for infectious disease: advances and challenges. Expert Rev Med Devices 2009; 6:641–651.

Kircher M, Kelso J. High-throughput DNA sequencing – concepts and limitations. Bioessays 2010; 32:524–536.

Layne SP, Beugelsdijk TJ. High-throughput laboratories for homeland and national security. Biosecur Bioterr 2003; 1:123–130.

Lehtoranta L *et al.* A novel, colorimetric neutralization assay for measuring antibodies to influenza viruses. J Virol Methods 2009; 159:271–276.

NIBSC. Current challenges in implementing cell-derived influenza vaccines: Implications for production and regulation. Vaccine 2007; 27:2907–2913.

Wang W *et al.* Establishment of retroviral pseudotypes with influenza hemagglutinins from H1, H3, and H5 subtypes for sensitive and specific detection of neutralizing antibodies. J Virol Methods 2008; 153:111–119.

WHO. Defining the safety profile of pandemic influenza vaccines. Lancet 2009; 375:9–11.

Wittman V *et al.* New tests for an old foe: an update on influenza screening. IDrugs 2010; 13:248–253.

Mathematical modelling

Ferguson NM, Galvani AP, Bush RM. Ecological and immunological determinants of influenza evolution. Nature 2003; 422:428–433.

Gog JR *et al.* Population dynamics of rapid fixation in cytotoxic T lymphocyte escape mutants of influenza A. Proc Natl Acad Sci USA 2003; 100:11143–11147.

Koelle K *et al.* Epochal evolution shapes the phylodynamics of interpandemic influenza a (H3N2) in humans. Science 2006; 314:1898–1903.

Plotkin JB, Dushoff J, Levin SA. Hemagglutinin sequence clusters and the antigenic evolution of influenza A virus. Proc Natl Acad Sci USA 2002; 99:6263–6268.

Rambaut A *et al.* The genomic and epidemiological dynamics of human influenza A virus. Nature 2008; 453:615–619.

Recker M *et al.* The generation of influenza outbreaks by a network of host immune responses against a limited set of antigenic types. Proc Natl Acad Sci USA 2007; 104:7711–7716.

WHO. Studies needed to address public health challenges of the 2009 H1N1 influenza pandemic: insights from modelling. The WHO Informal Network for Mathematical Modelling for Pandemic Influenza H1N1 2009 (Working

Group on Data Needs). PLoS Curr Influenza 2009 (revised 23 December 2009). RRN1135.

Annex 4: List of participants

William Kwabena Ampofo, National Influenza Centre, Accra, Ghana.

Novilia Sjari Bachtiar, PT. Bio Farma (Persero), Bandung, Indonesia.

Barnabas Bakamutumaho, National Influenza Centre, Entebbe, Uganda.

Ian Barr, WHO Collaborating Centre for Reference and Research on Influenza, Victoria, Australia.

Norman Baylor, Food and Drug Administration, Rockville, USA.

Trevor Bedford, Howard Hughes Medical Institute Associate, Ann Arbor, USA.

Janis Bernat, International Federation of Pharmaceutical Manufacturers & Associations (IFPMA), Geneva, Switzerland.

Simone Blayer, International Federation of Pharmaceutical Manufacturers & Associations (IFPMA), Marburg, Germany.

Mathilde Bourrier, Université de Genève, Geneva, Switzerland.

Cheikh Saad Bouh Boye, University Cheikh Anta Diop of Dakar, Dakar, Senegal.

Joe Bresee, Centers for Disease Control and Prevention (CDC), Atlanta, USA.

Eeva Broberg, European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden.

Maria Brytting, Swedish Institute for Infectious Disease Control, Olna, Sweden.

Doris Bucher, New York Medical College, Valhalla, USA.

Robin Bush, University of California Irvine, Irvine, USA.

Jorge Augusto Camara, Influenza and Respiratory Virus Laboratory, Cordoba, Argentina.

Frederick J. Cassels, National Institute for Health, Bethesda, USA.

Rajko Cebedzic, Medicines and Medical Devices Agency of Serbia, Republic of Serbia.

Mandeep Chadha, National Influenza Centre, Pune, India.

Malinee Chittakanpitch, National Institute of Health (NIH), Nonthaburi, Thailand.

Sarah Cobey, Harvard School of Public Health, Boston, USA.

Tony Colegate, International Federation of Pharmaceutical Manufacturers & Associations (IFPMA) Liverpool, UK.

Jaline Alves Cabral da Costa, Ministerio da Saude, Rio de Janeiro, Brazil.

Nancy J. Cox, Centers for Disease Control and Prevention (CDC), Atlanta, USA.

Sharon Daves, NAMRU-3, Cairo, Egypt.

Chris Detter, JGI-Los Alamos National Laboratory Center, Los Alamos, USA.

Minh Hung Do, Drug Administration of Vietnam, Ba Dinh District, Vietnam.

Lan Do Thi Diep, National Institute for Control of Vaccines and Biologicals, Hanoi, Vietnam.

Steven Edwards, Network of Expertise on Animal Influenzas (OFFLU) Steering Committee, Hereford, UK.

Maryna Eichelberger, Food and Drug Administration, Bethesda, USA.

Rajae El Aouad, Institute National d'Hygiène, Rabat, Morocco.

Nagwa El Kholy, Egyptian Organisation for Biological Products and Vaccines (VACSERA), Cairo, Egypt.

Othmar Engelhardt, National Institute for Biological Standards and Control (NIBSC), Potters Bar, UK.

Faten Fathalla, National Organization for Research and Control of Biologicals, Cairo, Egypt.

Neil Ferguson, Imperial College School of Medicine at St Mary's, London, UK.

John Franks, St Jude Children's Research Hospital, Memphis, USA.

Maria Y. Giovanni, National Institutes of Health, Bethesda, USA.

Julia Gog, Department of Applied Mathematics and Theoretical Physics, Cambridge, UK.

Gary Grohmann, Therapeutic Goods Administration Laboratories, Symonston, Australia.

Sunetra Gupta, University of Oxford, Oxford, UK.

Aissam Hachid, National Influenza Centre, Algiers, Algeria.

Alan Hay, National Institute for Medical Research, London, UK.

Jean-Michel Heraud, National Influenza Centre, Antananarivo, Madagascar.

Nurma Hidayati, National Agency of Drugs and Food Control, Jakarta, Indonesia.

Keith Howard, Baxter Innovations GmbH, Orth/Donau, Austria.

Sue Huang, Institute of Environmental Science and Research (ESR), Wellington, New Zealand.

Olav Hungnes, Norwegian Institute of Public Health, Oslo, Norway.

Daniel Jernigan, Centers for Disease Control and Prevention (CDC), Atlanta, USA.

Hervé Kadjo, Institut Pasteur de Côte d'Ivoire, Abidjan, Côte d'Ivoire.

Chun Kang, National Institute of Health, Korea CDC, Republic of Korea.

Jacqueline Katz, Centers for Disease Control and Prevention (CDC), Atlanta, USA.

Anne Kelso, WHO Collaborating Centre for Reference and Research on Influenza, Victoria, Australia.

Oleg I. Kiselev, National Influenza Center, St. Petersburg, Russian Federation.

Otfried Kistner, Baxter Innovations GmbH, Orth/Donau, Austria.

Alexander Klimov, Centers for Disease Control and Prevention (CDC), Atlanta, USA.

Kornnika Kullabutr, Ministry of Public Health, Nonthaburi, Thailand.

Linda Lambert, National Institutes of Health, Bethesda, USA.

Mai Le, National Institute of Hygiene and Epidemiology, Hanoi, Vietnam.

Patricia Leung-Tack, Sanofi Pasteur, France.

Roland Levandowski, Freelancer, Bethesda, USA.

Yan Li, Public Health Agency of Canada, Canada.

Wilina Lim, Centre for Health Protection, China, Hong Kong Special Administrative Region.

Oliver Kürsteiner Locher, Quality Control Berne, Berne, Switzerland.

Irma López-Martínez, INDRE (Instituto de Diagnostico y Referencia Epidemiologicos), México DF, Mexico.

Jaap Louwerens, Abbott Biologicals B.V., the Netherlands.

Maharani, Monovalent Vaccine Development, Bandung, Indonesia.

John McCauley, WHO Collaborating Centre for Reference and Research on Influenza, London, UK.

Kirill Mefed, Influenza Laboratory, Moscow, Russian Federation.

Joseph D. Miller, Centers for Disease Control and Prevention (CDC), Atlanta, USA.

A. C. Mishra, National Influenza Centre, Pune, India.

Talat Mokhtari-Azad, Iranian National Influenza Center, Tehran, the Islamic Republic of Iran.

Arnold Monto, University of Michigan School of Public Health, Ann Arbor, USA.

Karen Nelson, J. Craig Venter Institute, Rockville, USA.

Martha Nelson, National Institutes of Health, Bethesda, USA.

Elisabeth Neumeier, GlaxoSmithKline Biologicals, Germany.

Richard Njouom, NIC Cameroon, Yaoundé, Cameroon.

Walter O. Ochieng, National Influenza Centre, Nairobi, Kenya.

Takato Odagiri, National Institute of Infectious Diseases, Shinjuku, Japan.

Terezinha Maria de Paiva, Laboratorio de Virus Respiratorios, Sao Paulo, Brazil.

Pedro Pechirra, National Influenza Reference Laboratory, Lisbon, Portugal.

Leo Poon, The University of Hong Kong, China, Hong Kong Special Administrative Region.

Katarina Prosenc, NIC Slovenia, Ljubljana, Slovenia.

Andrew Rambaut, University of Edinburgh, Edinburgh, UK.

Endah Eny Riayati, National Quality Control Laboratory of Drug and Food (NQCLDF) Jakarta, Indonesia.

Guus Rimmelzwaan, Department of Virology, Rotterdam, the Netherlands.

Colin Russell, University of Cambridge, Cambridge, UK.

David Salisbury, Department of Health, London, UK.

Ondri Dwi Sampurno, Ministry of Health, Jakarta, Indonesia.

Vilma Savy, Instituto Nacional de Enfermdedades Infecciosas (ANLIS) C.G., Buenos Aires, Argentina.

Peter Schoofs, Commonwealth Serum Laboratories (CSL Limited), Victoria, Australia.

Brunhilde Schweiger, National Influenza Center, Berlin, Germany.

Paul Selleck, CSIRO Australian Animal Health Laboratory, Victoria, Australia.

Michael Shaw, Center for Disease Control and Prevention (CDC), Atlanta, USA.

David Shay, Centers for Disease Control and Prevention (CDC), Atlanta, USA.

Ahmad Sheibani, Ministry of Health and Medical Education (MOHME), Teheran, the Islamic Republic of Iran.

Marilda Siqueira, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil.

Amine Slim, National Influenza Centre, Tunis, Tunisia.

Derek Smith, University of Cambridge, Cambridge, UK.

David Spiro, J. Craig Venter Institute, Rockville, USA.

James Stevens, Centers for Disease Control and Prevention (CDC), Atlanta, USA.

Katharine M Sturm-Ramirez, National Institutes of Health, Bethesda, USA.

Yoshikazu Tada, The Research Foundation for Microbial Diseases of Osaka University, Kagawa, Japan.

Kazuyuki Takizawa, Denka Seiken Co. Ltd, Niigata, Japan.

Masato Tashiro, WHO Collaborating Centre for Reference and Research on Influenza, Tokyo, Japan.

Beverly Taylor, Novartis Vaccines and Diagnostics Ltd, Liverpool, UK.

Yves Thomas, National Centre of Influenza, Geneva, Switzerland.

Sylvie Van der Werf, Institut Pasteur, Paris, France.

Anthony L. Waddell, Freelancer, Stanley, UK.

Dayan Wang, Chinese Centre for Disease Control, Beijing, China.

Richard Webby, WHO Collaborating Centre for Studies on the Ecology of Influenza in Animals, Memphis, USA.

Stefan Weber, Bioscientia Medical Center Saarbruecken, Saarbruecken, Germany.

Jerry P. Weir, Center for Biologics Evaluation and Research/Food and Drug Administration, Bethesda, USA.

Ne Win, National Health Laboratory, Yangon, Myanmar.
John Wood, National Institute for Biological Standards and Control (NIBSC), Potters Bar, UK.
Aiping Wu, Chinese Academy of Sciences, Beijing, China.
Syed Sohail Zahoor Zaidi, National Institute of Health, Islamabad, Pakistan.
Maria Zambon, Health Protection Agency, London, UK.

WHO Secretariat

Cindy Aiello, Global Influenza Programme (GIP).
Claudia Alfonso, Immunization, Vaccines and Biologicals (IVB).
Terry Besselaar, Global Influenza Programme (GIP).
Rajesh Bhatia, WHO Regional Office for South-East Asia (SEARO).
Sylvie Briand, Global Influenza Programme (GIP).
Caroline Brown, WHO Regional Office for Europe (EURO).
Tristan Chevignard, Global Influenza Programme (GIP).
May Chin-May Chu, International Health Regulations (IHR).
Ellah Frodeman, Global Influenza Programme (GIP).
Keiji Fukuda, Health, Safety and Environment (HSE).
Christian Fuster, Global Influenza Programme (GIP).
Varja Grabovac, Global Influenza Programme (GIP).
Belinda Hall, Global Influenza Programme (GIP).
Hande Harmanci, Global Influenza Programme (GIP).
Anne Huvos, Global Influenza Programme (GIP).
Eileen Jameson, Health, Safety and Environment (HSE).
Harriete Jjuuko, Global Influenza Programme (GIP).
Marie-Paule Kieny, Immunization, Vaccines and Biologicals (IVB).
Maja Lièvre, Global Influenza Programme (GIP).
Jill Meloni, Health, Safety and Environment (HSE).
Angela Merianos, Global Alert and Response (GAR).
Anthony Mounts, Global Influenza Programme (GIP).
Elizabeth Mumford, Global Influenza Programme (GIP).
Otavio Oliva, WHO Regional Office for the Americas (AMRO).
Laszlo Palkonyay, Immunization, Vaccines and Biologicals (IVB).
Javier Penalver Herrero, Global Influenza Programme (GIP).
Helena Rebelo de Andrade, Global Influenza Programme (GIP).
Cathy Roth, Health, Safety and Environment (HSE).
Nahoko Shindo, Global Influenza Programme (GIP).
Ludy Suryantoro, Health, Safety and Environment (HSE).
Niteen Wairagkar, Global Influenza Programme (GIP).
David John Wood, Immunization, Vaccines and Biologicals (IVB).
Wenqing Zhang, Global Influenza Programme (GIP).

Acknowledgements

Expansion of the Global Measles and Rubella Laboratory Network 2005–09

David A. Featherstone,[1,a] Paul A. Rota,[2] Joseph Icenogle,[2] Mick N. Mulders,[3] Youngmee Jee,[4] Hinda Ahmed,[5] Ana Maria Bispo de Filippis,[6] Nalini Ramamurty,[7] Eugene Gavrilin,[3] Charles Byabamazima,[8] Annick Dosseh,[9] Wenbo Xu,[10] Katsuhiro Komase,[11] Masato Tashiro,[11] David Brown,[12] William J. Bellini,[2] and Peter Strebel[1]

[1]Department of Immunization, Vaccines and Biologicals, Family and Community Health Cluster, World Health Organization (WHO), Geneva, Switzerland; [2]Centers for Disease Control and Prevention, Atlanta, Georgia; [3]WHO Regional Office for Europe, Copenhagen, Denmark; [4]WHO Regional Office for the Western Pacific, Manila, Philippines; [5]WHO Regional Office for the Eastern Mediterranean, Cairo, Egypt; [6]PAHO, Washington, DC; [7]WHO SEARO, Delhi, India; [8]WHO AFRO, Harare, Zimbabwe; [9]WHO Regional Office for Africa, Ouagadougou, Burkina Faso; [10]National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, China; [11]Department of Virology, National Institute of Infectious Diseases, Tokyo, Japan; and [12]Health Protection Agency, London, United Kingdom

Enhancing measles surveillance with integration of epidemiologic and laboratory information is one of the key strategies for accelerated measles control and elimination. The World Health Organization (WHO) Global Measles and Rubella Laboratory Network (LabNet) has been developed since 2000 to currently include 690 laboratories serving 183 countries. The LabNet testing strategy follows well-validated, standardized procedures for confirming suspected cases and for monitoring measles and rubella virus transmission patterns. The strength of the LabNet is a strong quality assurance program that monitors the performance of all laboratories through annual proficiency testing and continuous assessment. In the 5-year period 2005–2009, the results of >1 million measles immunoglobulin M (IgM) tests have been reported by the LabNet and, in addition, sequence information on >7000 measles and 600 rubella viruses has been shared. Progress with the development of the LabNet during 2005–2009 is discussed.

Progress with measles control since the year 2000 has been dramatic. It is estimated that >4.5 million measles deaths have been prevented as of 2008 through implementation of the strategies developed by WHO and the United Nations Children's Fund [1]. Four World Health Organization (WHO) regions (Americas [AMR], Eastern Mediterranean [EMR], European [EUR] and Western Pacific [WPR]) have established elimination goals, and the WHO African Region (AFR) has developed strategies leading to an elimination goal that they term "pre-elimination." The Southeast Asian Region (SEAR) has a mortality reduction goal.

Enhancing measles surveillance with the integration of epidemiologic and laboratory information is one of the key strategies for accelerated measles control and elimination, and the WHO Measles and Rubella Laboratory Network (LabNet) has been established to provide timely and accurate data for the surveillance of measles and rubella.

FUNCTION OF THE GLOBAL MEASLES AND RUBELLA LABORATORY NETWORK

The LabNet plays a critical role in confirming suspected cases of measles through the detection of specific immunoglobulin M (IgM) and testing measles-negative cases for evidence of rubella infection. In addition, identification and characterization of measles and

Potential conflicts of interest: none reported.

Supplement sponsorship: This article is part of a supplement entitled "Global Progress Toward Measles Eradication and Prevention of Rubella and Congenital Rubella Syndrome," which was sponsored by the Centers for Disease Control and Prevention.

Presented in part: WHO 8th Global Measles and Rubella Laboratory Network meeting, 20–22 September 2010. This is the first manuscript to describe an update of the WHO LabNet for the period 2005–09.

[a]The author is a staff member of the World Health Organization. The author alone is responsible for the views expressed in this publication and they do not necessarily represent the decisions, policy, or views of the World Health Organization.

Correspondence: David A. Featherstone, BSc, Expanded Programme on Immunization, Department of Immunization, Vaccines and Biologicals, World Health Organization, Avenue Appia 20, Geneva 1211, Switzerland (featherstoned@who.int).

The Journal of Infectious Diseases 2011;204:S491–S498

0022-1899 (print)/1537-6613 (online)/2011/204S1-0063$14.00
DOI: 10.1093/infdis/jir107

rubella viruses provide information about the origin of outbreaks, whether endemic or imported, which is required for monitoring progress toward elimination.

As disease prevalence falls, medical personnel become less experienced in recognizing clinical measles and rubella, thus increasing the importance of laboratory methods to distinguish measles and rubella from other diseases with similar clinical presentations. The strategy of testing measles-negative cases for rubella was developed primarily to enhance the measles surveillance system in the WHO Region of the Americas. This strategy revealed previously unrecognized widespread circulation of rubella virus in many countries in the region. Furthermore, various studies indicated that the disease burden of congenital rubella syndrome was significant in the Americas and underscored significant morbidity from rubella and congenital rubella syndrome in the Western hemisphere [2–4]. After measles elimination was accomplished in the AMR in 2002, a rubella elimination program was established using the same strategies, including laboratory-based surveillance.

As of July 2010, the LabNet consists of 690 laboratories, all of which follow a standardized set of testing protocols and reporting procedures with a strong focus on quality assurance. The structure of the LabNet was based on the successful polio laboratory network [5] and was established in 4 tiers of laboratories: national level (n = 162), regional reference (n = 19), global specialized (n = 3), and subnational level (n = 506) (Figure 1).

The cornerstones of the LabNet are the national-level laboratories, which provide timely and accurate case confirmation of suspected measles and/or rubella cases using standard enzyme-linked immunosorbent assay (ELISA)-based IgM detection from serum samples collected at first contact with the health system. These laboratories are closely linked with the national disease control programs and often are responsible for surveillance of other exanthematous diseases as well, including dengue, parvovirus B19, and human herpesvirus type 6. Differential testing for other exanthematous disease can improve the interpretation of measles and rubella test results, especially in low-incidence settings. By June 2010, 160 of the 193 member states of WHO had established national laboratories with a further 24, mostly countries with small populations, served by another country's laboratory. Some large countries or those with logistical challenges in transporting samples have established laboratories at the subnational level to facilitate the timely testing and reporting of results. For example, China has established 362 laboratories at the first (n=31) and second (n=331) administrative levels (provincial and prefecture, respectively) [6]. In 2009, the Chinese laboratory network tested more than 86,000 serum samples for measles and rubella IgM antibodies following the same testing strategies and comprehensive quality assurance and accreditation assessments as the remainder of the global LabNet.

Two or three regional reference laboratories have been selected in each WHO region. The regional reference laboratories are regional centers of excellence that support the capacity building of the national-level laboratories under their

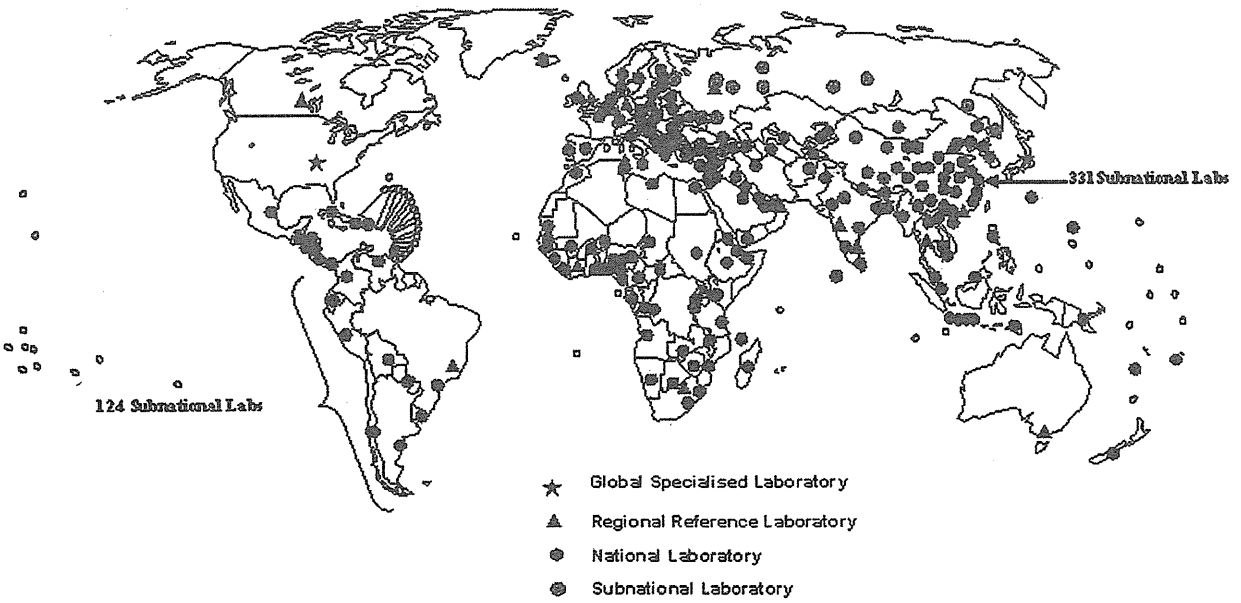

Figure 1. Location of laboratories belonging to the Measles and Rubella LabNet.

responsibility and provide confirmatory testing of a proportion of the samples tested by the national-level laboratories. They also support genetic characterization of circulating wild-type viruses to help monitor viral transmission pathways and progress toward measles elimination. Global specialized laboratories are global centers of excellence that have been established in Japan, the United Kingdom, and the United States. The global specialized laboratories contribute to the standardization of procedures and protocols, development and validation of new techniques, and global capacity building through focused training, and some provide financial support.

LABORATORY TESTING STRATEGIES

The standard LabNet test for measles and rubella confirmation is the detection of IgM in serum, oral fluid, or eluates of dried blood spots collected from suspected cases at their first contact with a health facility [7]. For molecular testing, preferred samples are throat swabs or oral fluid for direct virus detection of viral RNA by reverse-transcription polymerase chain reaction assay. Throat swab samples are also used to isolate measles virus in cell culture. Urine samples and peripheral blood mononuclear cells can also be used for virus culture, although in practice these have proven to be more difficult to collect, to transport, and to process successfully. The LabNet has introduced the Vero/hSLAM cell line as the standard for virus isolation, provided by Dr Yanagi [8]. This cell line was demonstrated to be sensitive for both wild-type measles and rubella virus culture [9](unpublished data from LabNet) and has proven to be easily handled in the LabNet.

ELISA-based IgM detection in serum was established as an accurate, simple, and rapid procedure to confirm cases of suspected measles or rubella. Various commercial assays were comprehensively validated [10], and assays with high sensitivity and specificity are currently used by most national laboratories in the LabNet. National and subnational laboratories in some countries use locally produced commercial assays. Locally produced assays in the United States, the Netherlands, Japan [11, 12], China, and the Russian Federation have been evaluated (unpublished data from LabNet), some of which have shown sensitivity and specificity comparable with the commercial assays used by most laboratories in the LabNet.

A serum sample collected at first contact with a health facility is considered the gold standard for measles IgM ELISA detection, because sensitivity has been reported to be 77% within 72 hours after rash onset and 100% at 4 days [13]. Oral fluid and dried blood samples have been used for enhancing the diagnosis of numerous diseases [14–20] and have been considered for use in the LabNet. The LabNet has comprehensively evaluated oral fluid and dried blood sampling techniques, using IgM detection for measles and rubella diagnosis and viral RNA detection and sequencing for molecular surveillance, determining their temperature stability outside the cold chain, and assessing their ease of use [21, 22]. In 2010, five African countries (Benin, Cote d'Ivoire, Kenya, Malawi, and Zimbabwe) began evaluating oral fluid sampling techniques as a mechanism to enhance surveillance through use of a single sample for both case confirmation (IgM) and molecular surveillance (RNA). If results from the trial are encouraging, this technique will be considered for introduction to a larger number of countries in the region. Several countries within the WHO Southeast Asian Region are also considering trying the use of oral fluid samples to enhance surveillance.

QUALITY ASSURANCE AND SURVEILLANCE INDICATORS

Quality assurance is a major strength of the LabNet and includes confirmatory testing of national and subnational laboratory results, a global proficiency testing program, implementation of in-house quality control measures, and an accreditation program. The global proficiency testing program for the LabNet began in 2000, and initially 38 laboratories were involved [23]. The proficiency testing serum panels consist of 20 samples from patients with recent measles, rubella, or other exanthema and are produced and analyzed by the Victorian Infectious Diseases Reference Laboratory in Melbourne, Australia, one of the LabNet's regional reference laboratories for the Western Pacific Region. In 2009, there were 220 laboratories participating in the proficiency testing program, including all national, regional reference, and global specialized laboratories, the 31 Chinese provincial laboratories, and all subnational laboratories in the Russian Federation, Turkey, and Ukraine. For measles, 98% of laboratories achieved a passing score ($\geq$90%), with 84% of laboratories achieving 100%. For rubella, the scores were 99% and 94%, respectively.

Because the proficiency testing program provides a snapshot of the accuracy of IgM testing, a continual assessment of a laboratory's testing accuracy was established through a confirmatory testing program. This program requires national and subnational laboratories to forward annually a representative sample (approximately 10% of all samples) to the designated regional reference laboratory for repeat testing with an expected concordance of $\geq$90%. The WHO accreditation program monitors performance of laboratories on a continual basis, but the assessment is expected to be completed annually. Seven performance criteria are assessed: reporting timeliness of case confirmation results, reporting timeliness of genetic characterization data, accuracy of results compared with the reference laboratory, implementation of specified quality control procedures, a passing score in the annual proficiency test, a minimum number of 50 tests performed per year, and a passing score on a comprehensive onsite review of the laboratory's work practices. National laboratories and reference laboratories have similar

criteria, but the expected passing scores are higher for the reference laboratories.

The function of the accreditation program is not only to monitor the quality of the LabNet but also, and most importantly, to identify any capacity building that is needed and to troubleshoot any problems that may have arisen. A laboratory that achieves less than the passing score on any one of the performance criteria will work with the WHO regional laboratory coordinator to identify areas in which improvement is needed and to develop and to implement a workplan to reach the full accreditation benchmarks as soon as possible. Quality assurance of serologic testing is comprehensive, and plans have been developed for a similar program to ensure the accuracy of molecular techniques and the sensitivity of virus culture.

A number of the surveillance indicators that are used to assess the quality of measles surveillance are dependant on laboratory function. These indicators include the percentage of cases with an adequate sample, the percentage of samples that arrive in the laboratory within 3 days, the percentage of results reported within 7 days, and the percentage of suspected cases discarded by the laboratory. LabNet member laboratories have achieved the target of 80% for most of these indicators; however, in some regions obtaining adequate samples is difficult because of logistic problems or inadequate case investigations, and often timely transportation of samples to the laboratory is difficult to achieve.

LABNET COORDINATION

Coordination of the LabNet is a key facet to a successfully functioning network. Communication among all levels of laboratories is carried out through regular meetings, workshops, and information exchange through telephone and email. Global LabNet meetings have been held annually since 2001, and regional laboratory coordination meetings are held in each of the 6 WHO Regions every 1–3 years. Each of the 6 WHO Regions has a dedicated laboratory coordinator with expertise in virology and experience with managing laboratory networks. These coordinators are positioned in the regional or subregional offices, and one is located in WHO headquarters. Their primary roles are to work with Ministries of Health to assess and to select appropriate laboratories, to expand the capacity and capability of LabNet through standardized training activities, to ensure laboratories are provided with appropriate equipment, to provide standardized supplies, to establish regular reporting structures, to perform accreditation reviews, and to coordinate the resolution of any performance issues. The regional reference and global specialized laboratories play a key role in strengthening capacity through support of training, providing troubleshooting advice, and developing and validating new technologies to improve laboratory-based surveillance.

LABNET INTEGRATION AND CAPACITY BUILDING

The LabNet was built on the experience gained from establishing the polio laboratory network and capitalized on the same infrastructure in a number of countries. In turn, the measles and rubella LabNet has been used to build similar laboratory-based surveillance networks globally. As of June 2010, these include laboratories focusing on yellow fever in West and Central Africa (n=23), Japanese encephalitis in SEAR (n=13) and WPR (n=9) (Figure 1), and human papillomavirus infection (n=10) globally. The rotavirus laboratory network was established in the late 1990s in WPR and SEAR and extended to all WHO regions by 2007, using a similar proficiency testing and data sharing model [24–26]. The pediatric bacterial meningitis laboratory network for *Haemophilis influenzae* type b surveillance, dating from the early 2000s in the African region, used a similar proficiency testing and data sharing program. There is considerable overlap in personnel, laboratories, and institutions used for these different surveillance activities. A number of measles LabNet personnel have also been involved in the enhanced global surveillance for pandemic influenza and in establishing routine surveillance for seasonal influenza. The more recently initiated global disease surveillance programs benefit from the capacity developed in establishing the polio and measles and rubella LabNets, especially through the linkages developed with WHO and specialized laboratories, the provision of standardized equipment and supplies, and the skills developed through the extensive training programs implemented.

The capacity of the Measles and Rubella LabNet has developed rapidly, especially in the past 5 years. The number of samples tested for measles IgM has increased from 119,000 in 2005 to 173,000 in 2009, with most of the increase in the Western Pacific Region. The number of samples from SEAR is expected to increase considerably after India begins case-based surveillance (Figure 2). Approximately 67% of all samples were also tested for rubella IgM, with approximately 400,000 IgM tests being performed in total by the LabNet in 2008. In 2009, there were 193,120 samples received for testing in the LabNet, of which 327,082 tests were performed for measles and/or rubella IgM antibodies (Table 1).

For monitoring and tracking molecular surveillance data, genotype databases for measles and rubella were established at WHO headquarters in 2006, which include genotype and basic epidemiologic data. As of 1 June 2010, 7675 measles viruses from 123 countries have been sequenced and key variables submitted to this database, and details on 600 rubella viruses from 36 countries have been submitted [27, 28]. In 2008, a joint Health Protection Agency and WHO measles sequence database (MeaNS) was also established, which included sequences from 4,200 viruses by 1 June 2010.

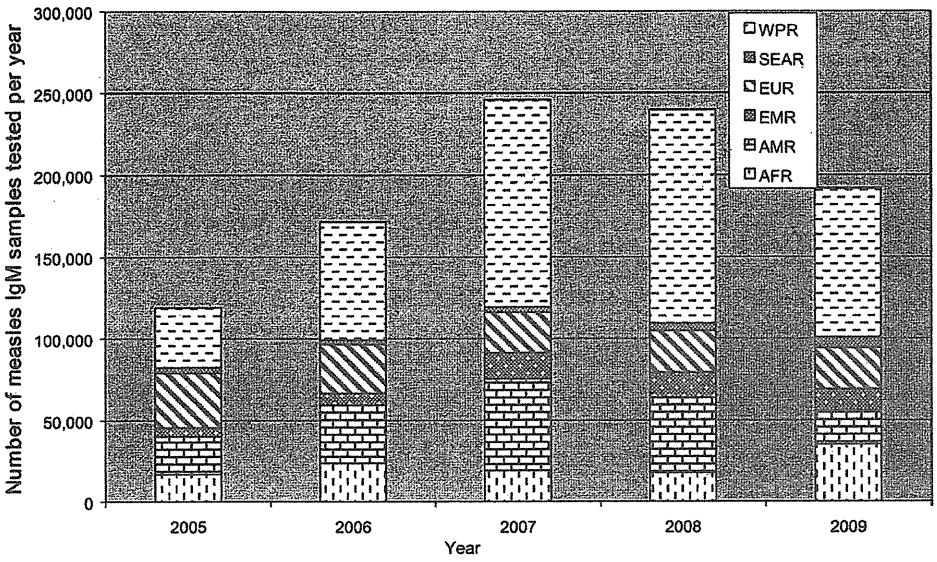

Figure 2. Measles immunoglobulin M (IgM) samples tested per year, by World Health Organization region, 2005–09. Two large countries, India and Pakistan, have yet to establish case-based surveillance. AFR, African Region; AMR, Americas Region; EMR, Eastern Mediterranean Region; EUR, European Region; SEAR, Southeast Asian Region; WPR, Western Pacific Region.

RESOURCES

Resources for the LabNet are provided largely through bilateral support mechanisms. Countries provide the infrastructure costs and cover staff salaries and overheads; and WHO, with the aid of its partners—a key one of which is the US Centers for Disease Control and Prevention—supports training activities and assists laboratories in obtaining optimal levels of equipment and supplies [29]. In some WHO Regions, operational costs may be provided to priority countries in addition to supplies and training.

DISCUSSION

The development of LabNet has progressed rapidly during the past 10 years. The number of laboratories has increased from 60 in 2000 to 690 in 2010 but has likely reached a plateau, because almost all countries have established laboratory-based measles surveillance. However, the complexity of testing will continue to increase as molecular surveillance to determine transmission patterns and to distinguish between imported and endemic measles cases is fully embraced. LabNet testing is critical for adding specificity to surveillance information when incidence

Table 1. Reported Laboratory Workload and Performance of Laboratories Participating in the LabNet by World Health Organization (WHO) Region, 2009

		Measles			Rubella			
WHO Region	No. of serum samples received	No. of samples tested	No. of samples IgM positive	% Positive	No. of samples tested	No. of samples IgM positive	% Positive	Results reported within 7 days,[a] %
AFR	36,558	35,242	10,088	29	29,611	5157	17	59
AMR	20,668	20,415	89	0.4	20,415	18	0.09	84
EMR	13,222	13,222	5950	45	11,824	770	7	95
EUR	24,929	18,665	3314	18	17,660	1094	6	78
SEAR	6561	6128	1103	18	5368	2656	49	70
WPR	91,182	71,426	34,412	48	49,084	12,787	26	>90
Total	**193,120**	**165,098**	**54,956**	**33**	**133,962**	**22,482**	**17**	

NOTE. AFR, African Region; AMR, Americas Region; EMR, Eastern Mediterranean Region; EUR, European v; SEAR, Southeast Asian Region; WPR, Western Pacific Region.

[a] For the AMR, percentage of results reported within 4 days.

decreases and the predictive value of clinical diagnosis diminishes. In addition, monitoring progress toward elimination through sequence analysis of currently circulating wild-type viruses is needed to determine whether a virus is endemic or imported.

Despite the large improvement in molecular surveillance, gaps in baseline molecular surveillance for measles still exist and no genotype data are available for 72 countries, although 25 of these are from the Region of the Americas, which has already eliminated measles, and 20 are small countries with a very low incidence of measles. Gathering information on baseline rubella virus sequence data is still at an early stage of development, with no data reported from 158 countries.

As countries progress toward elimination of measles, laboratories will be required to provide genotype information from 80% of the chains of transmission. Achieving this target will require close coordination between those conducting case investigations and the LabNet and will increase the number of samples that must be processed and tested by the laboratory. Also, in elimination or pre-elimination settings, rapid confirmation of rashes due to vaccine reactions using molecular techniques will become increasingly important.

The positive predictive value of the procedure of using a single IgM ELISA on serum for confirming suspected measles and rubella cases in the LabNet is high when incidence of disease is high, but concerns have been raised as to whether these testing procedures are appropriate under low-incidence settings, because the positive predictive value decreases significantly [30]. Although the WHO Region of the Americas has successfully documented elimination of both measles and rubella using these testing strategies, the LabNet is investigating procedures that may improve the positive predictive value in low-incidence settings. In addition, specialized serologic testing, such as IgG avidity assays and plaque-reduction neutralization assays, are used in more advanced laboratory settings to aid in the classification of more difficult cases that sometimes arise in low-incidence settings [31, 32].

CHALLENGES

As of 2009, only 12 (Algeria, Comoros, Guinea Bissau, India, Mauritius, Monaco, Morocco, Pakistan, San Marino, Sao Tome and Principe, Seychelles, and Somalia.) of the 193 member states of WHO have yet to implement case-based surveillance with laboratory confirmation; however, 2 are large-population countries: India and Pakistan. However, LabNet laboratories are performing measles outbreak confirmation in all 12 except Mauritius, San Marino, Sao Tome and Principe, and Seychelles. The LabNet has shown that it has the capacity to conduct up to 400,000 IgM tests annually (2008) for measles and rubella, and individual laboratories have responded to an almost 30-fold increase in weekly baseline testing rates. When all countries establish case-based surveillance and meet the minimum surveillance and testing criteria, the annual number of LabNet tests will increase by an estimated 70,000. The LabNet has the capacity to meet this increased throughput; however, finding the resources to procure additional kits and to train and to equip additional laboratories in the countries that have yet to meet the surveillance indicators will require increased advocacy efforts and more country commitment to supporting measles and rubella laboratory-based surveillance.

Despite progress in measles control, large measles outbreaks occurred in 2009 and 2010 in Bulgaria and several African countries. Testing all suspected cases during large outbreaks is not essential if countries follow WHO's recommended guidelines to epidemiologically link suspected cases to laboratory-confirmed cases [33]. However, some countries continue to collect samples from almost all suspected cases during an outbreak, often increasing the laboratory's workload by many-fold. For example, in 2009 South Africa experienced a large outbreak of measles that saw the national laboratory reporting almost 17,000 positive cases during a 12-month period starting in mid-2009 [34]. The laboratory tested approximately 50 serum samples per week before the outbreak but progressed to testing >1400 specimens per week at the peak of the outbreak, within a period of several months. Although the national laboratory performed exceptionally well in managing the heavy workload, it was put under considerable pressure to provide an acceptable turn-around time while at the same time maintaining high-quality results. Laboratories in other countries, including Botswana, Cambodia, Lesotho, Malawi, Philippines, Swaziland, Ukraine, Viet Nam, and Zimbabwe, have experienced a large increase in workload following outbreaks. Although the weekly increase in number of samples tested was less than in South Africa, the impact on increasing the reporting turnaround time has been similar. Procuring sufficient test kits, maintaining quality, and timely reporting of results under these circumstances make a considerable challenge.

Some countries have a considerable number of private laboratories in the health sector that are used for testing suspected measles cases. Many of these laboratories are funded through health insurance and may test only for the specific diseases covered by the reimbursement. It has been reported from the European and Western Pacific regions that the large number of these laboratories in some countries makes it difficult to monitor the quality and accuracy of test procedures and to ensure regular reporting through the public health system. In the Region of the Americas, the United States reported that approximately 25,000 measles IgM tests were performed by private laboratories in 1997 [35]. External monitoring of quality control in commercial laboratories is a challenge, although in some cases, LabNet laboratories provide confirmatory and follow-up testing procedures.

Table 2. Laboratory-Based Training Workshops Held January 2009–June 2010 for Measles, Rubella, Yellow Fever, and Japanese Encephalitis Surveillance

Region	No. of countries	No. of laboratories participating	No. of participants
AFR	24	26	38
EMR	11	12	18
SEAR	11	18	40
WPR	14	46	82
Total	60	102	178

NOTE. AFR, African Region; EMR, Eastern Mediterranean Region; SEAR, Southeast Asian Region; WPR, Western Pacific Region.

As part of the quality assurance program, national and subnational laboratories are required to send a proportion of their IgM samples to their designated reference laboratory for confirmatory testing. The international shipment for these procedures has amounted to a considerable cost burden for some countries and has posed multiple logistic difficulties when samples are transported across national borders. The LabNet has validated the use of drying serum onto filter paper for temperature-stable shipment, thus reducing the cost of shipping under refrigeration temperature. Furthermore, dried serum samples are approved as exempt from dangerous goods requirements and regulations [36].

The LabNet has strengthened the health system by building capacity, strengthening laboratory infrastructure, and developing human resources. In the 18 months from 2009 to June 2010, >170 national and reference laboratory staff were trained to perform IgM ELISA procedures and polymerase chain reaction and sequencing techniques, and to improve data and laboratory management for measles, yellow fever, and Japanese encephalitis surveillance (Table 2). However, as countries strive to reach their measles elimination goals, partnership support for the LabNet must increase as the complexity of testing and number of samples increase.

Funding

The development of the LabNet is supported in part by a grant (CDC PMS U66/IP000161-03) from the Centers for Disease Control and Prevention.

References

1. WHO; UNICEF. Vaccine preventable deaths and the global immunization vision and strategy, 2006–2015. MMWR Morb Mortal Wkly Rep **2006**; 55:511–5.
2. Cutts FT, Vynnycky E. Modelling the incidence of congenital rubella syndrome in developing countries. Int J Epidemiol **1999**; 28:1176–84.
3. Rittler M, López-Camelo J, Castilla EE. Monitoring congenital rubella embryopathy. Birth Defects Res A Clin Mol Teratol **2004**; 70:939–43.
4. World Health Organization. Progress towards eliminating rubella and congenital rubella syndrome in the Western hemisphere, 2003–2008. Wkly Epidemiol Rec **2008**; 83:395–400.
5. Featherstone DA, Brown DWG, Sanders R. Development of the global measles laboratory network. J Infect Dis **2003**; 187:S264–9.
6. Zhang Y, Ji Y, Jiang X, et al. Genetic characterization of measles viruses in China, 2004. Virol J **2008**; 5:120.
7. WHO. Laboratory manual for laboratory diagnosis of measles and rubella infection, 2nd ed. WHO/IVB/07.01, **2006**.
8. Ono N, Tatsuo H, Hidaka Y, Aoki T, Minagawa H, Yanagi Y. Measles viruses on throat swabs from measles patients use signaling lymphocytic activation molecule (CDw150) but not CD46 as a cellular receptor. J Virol **2001**; 75:4399–401.
9. Keniscope C, Juliana R, Subri H, et al. Isolation of measles virus from clinical specimens using B95a and Vero/hSLAM cell-lines. Med J Malaysia **2009**; 64:37–40.
10. Ratnam S, Tipples G, Head C, Fauvel M, Fearon M, Ward BJ. Performance of indirect immunoglobulin M (IgM) serology tests and IgM capture assays for laboratory diagnosis of measles. J Clin Microbiol **2000**; 38:99–104.
11. Tipples GA, Hamkar R, Mohktari-Azad T, et al. Assessment of immunoglobulin M enzyme immunoassays for diagnosis of measles. J Clin Microbiol **2003**; 41:4790–2.
12. Tipples GA, Hamkar R, Mohktari-Azad T, et al. Evaluation of rubella IgM enzyme immunoassays. J Clin Virol **2004**; 30:233–8.
13. Helfand RF, Heath JL, Anderson LJ, Maes ER, Guris D, Bellini WJ. Diagnosis of measles virus with an IgM capture EIA: the optimal timing of specimen collection after rash onset. J Infect Dis **1997**; 175:195–9.
14. Ibrahim SA, Abdallah A, Saleh EA, Osterhaus AD, De Swart RL. Measles virus-specific antibody levels in Sudanese infants: a prospective study using filter paper blood samples. Epidemiol Infect **2006**; 134:79–85.
15. Karapanagiotidis T, Riddell M, Kelly H. Detection of rubella immunoglobulin M from dried venous blood spots using a commercial enzyme immunoassay. Diagn Microbiol Infect Dis **2005**; 53:107–11.
16. Tran TN, de Vries PJ, Hoang LP, et al. Enzyme-linked immunoassay for dengue virus IgM and IgG antibodies in serum and filter paper blood. BMC Infect Dis **2006**; 25:6–13.
17. Amado LA, Villar LM, de Paula VS, de Almeida AJ, Gaspar AM. Detection of hepatitis A, B, and C virus-specific antibodies using oral fluid for epidemiological studies. Mem Inst Oswaldo Cruz **2006**; 101:149–55.
18. Forbi JC, Obagu JO, Gyar SD, Pam CR, Pennap GR, Agwale SM. Application of dried blood spot in the sero-diagnosis of hepatitis B infection (HBV) in an HBV hyper-endemic nation. Ann Afr Med **2010**; 9:44–5.
19. Amado LA, Villar LM, de Paula VS, Gaspar AM. Comparison between serum and saliva for the detection of hepatitis A virus RNA. J Virol Methods **2008**; 148:74–80.
20. Vijaylakshmi P, Muthukkaruppan VR, Rajasundari A, et al. Evaluation of a commercial rubella IgM assay for use on oral fluid samples for diagnosis and surveillance of congenital rubella syndrome and postnatal rubella. J Clin Virol **2006**; 37:265–8.
21. WHO. Measles and rubella laboratory network: 2007 meeting on use of alternative sampling techniques for surveillance. Wkly Epidemiol Rec **2008**; 83:225–32.
22. Recommendations from an ad hoc meeting of the WHO measles and rubella laboratory network (LabNet) on use of alternative diagnostic samples for measles and rubella surveillance. MMWR Morb Mortal Wkly Rep **2008**; 57:657–60.
23. Stambos V, Leydon J, Riddell M, et al. Evaluation of global measles and rubella quality assurance program, 2001 to 2008. J Infect Dis. 2011. In press.
24. Bresee JS, Hummelman E, Nelson EA, Glass RI. Rotavirus in Asia: the value of surveillance for informing decisions about the introduction of new vaccines. J Infect Dis **2005**; 192(suppl);S1–5.
25. Widdowson MA, Steele AD, Vodjani J, Wecker J, Parashar UD. Global rotavirus surveillance: determining the need and measuring the impact of rotavirus vaccines. J Infect Dis **2009**; 200(suppl);S1–8.

26.2 CDC. Rotavirus surveillance—worldwide, 2001–2008. MMWR Morb Mortal Wkly Rep **2008**; 57:1255–7.
27. Rota PA, Brown K, Mankertz A, et al. Global distribution of measles genotypes and measles molecular Epidemiology. J Infect Dis Measles Supplement **2011.** In press.
28. Rota PA, Brown K, Hübschen JM, et al. Improving global virologic surveillance for measles and rubella. J Infect Dis Measles Supplement **2011.** In press.
29. WHO. Update: global measles and rubella laboratory network, 2003–2005. Wkly Epidemiol Rec **2005**; 80:377–88.
30. Bellini WJ, Helfand RF. The challenges and strategies for laboratory diagnosis of measles in an international setting. J Infect Dis **2003**; 187(suppl 1);S283–90.
31. Hickman CJ, Hyde TB, Sowers SB, et al. Laboratory characterization of measles virus infection in previously vaccinated and unvaccinated individuals. J Infect Dis Measles Supplement **2011**; 203:S551–S560.
32. Dietz V, Rota J, Izurieta H, Carrasco P, Bellini W. The laboratory confirmation of suspected measles cases in settings of low measles transmission: conclusions from the experience in the Americas. Bull World Health Organ **2004**; 82:852–7.
33. WHO. WHO recommended standards for surveillance of selected vaccine-preventable diseases. WHO/V&B/03.01, 2003.
34. National Institute for Communicable Diseases (a division of the National Health Laboratory Service) laboratory-confirmed measles report. http://www.nicd.ac.za/measles_out/measles_current.pdf. Accessed 29 September 2010.
35. Harpaz R, Papania MJ. Can a minimum rate of investigation of measles-like illnesses serve as a standard for evaluating measles surveillance? J Infect Dis **2004**; 190:2195–6.
36. WHO guidance on regulations for the transport of infectious substances, 2007–2008. http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_EPR_2007_2/en/index.html Accessed 29 September 2010.

Fujitsuka et al. BMC Infectious Diseases 2011, 11:168
http://www.biomedcentral.com/1471-2334/11/168

BMC Infectious Diseases

RESEARCH ARTICLE Open Access

A molecular epidemiological study of respiratory viruses detected in Japanese children with acute wheezing illness

Asako Fujitsuka[1,2], Hiroyuki Tsukagoshi[3], Mika Arakawa[4], Kazuko Goto-Sugai[1,2], Akihide Ryo[5], Yoshimichi Okayama[6], Katsumi Mizuta[7], Atsuyoshi Nishina[8], Masakazu Yoshizumi[3], Yoichi Kaburagi[1], Masahiro Noda[9], Masato Tashiro[10], Nobuhiko Okabe[11], Masaaki Mori[2], Shumpei Yokota[2] and Hirokazu Kimura[3,11*]

Abstract

Background: Recent studies strongly suggest that some respiratory viruses are associated with the induction of acute wheezing and/or exacerbation of bronchial asthma. However, molecular epidemiology of these viruses is not exactly known.

Methods: Using PCR technology, we attempted to detect various respiratory viruses from 115 Japanese children. Furthermore, the detected viruses were subjected to homology, pairwise distance, and phylogenetic analysis.

Results: Viruses were detected from 99 (86.1%) patients. Respiratory syncytial virus (RSV) alone and human rhinovirus (HRV) alone were detected in 47 (40.9%) and 36 (31.3%) patients, respectively. Both RSV and HRV were detected in 14 (12.2%) patients. Human metapneumovirus (HMPV) alone and human parainfluenza virus (HPIV) alone were detected in 1 (0.9%) patient each, respectively. Homology and phylogenetic analyses showed that the RSV and HRV strains were classified into genetically diverse species or subgroups. In addition, RSV was the dominant virus detected in patients with no history of wheezing, whereas HRV was dominant in patients with a history of wheezing.

Conclusions: The results suggested that these genetically diverse respiratory viruses, especially RSV and HRV, might be associated with wheezing in Japanese children.

Background

A range of respiratory viruses are known to cause acute respiratory infections (ARI), including the common cold, bronchiolitis, and pneumonia in humans [1]. The major pathogens are potentially respiratory syncytial virus (RSV), human rhinovirus (HRV), human metapneumovirus (HMPV), human parainfluenza virus (HPIV), enterovirus (EV), influenza viruses (InfV), adenoviruses (AdV), and human bocavirus (HBoV) [2,3]. Respiratory infections by RSV, HRV, and HPIV are implicated in the induction of wheezing and the exacerbation of asthma, although their mechanisms are not clearly known [4]. The prevalence of asthma in developed countries is around 10 to 15% in children, while the prevalence is lower but increasing rapidly in developing countries [5]. Accumulating evidence indicates that the etiology of most cases of asthma, namely virus-induced asthma, is linked to such respiratory virus infections [6-9]. In addition, other epidemiological studies suggest that about 70% of infants have experienced an RSV infection by the age of 1 year, and 100% by the age of 2 years; the host response to the virus varies greatly, but includes upper respiratory tract infections, typical bronchiolitis (with crepitations but no wheeze), and RSV-induced wheezy bronchitis [10,11]. In addition, HRV includes over 100 serotypes and most of these are epidemic, although their epidemiology is not known [12]. Similarly, most children are infected at least once with HPIV early in life, but reinfections occur throughout life [13]. HBoV and HMPV are recently discovered

* Correspondence: hkimura0511@gmail.com
[3]Department of Health Science, Gunma Prefectural Institute of Public Health and Environmental Sciences, 378 Kamioki-machi, Maebashi-shi, Gunma 371-0052, Japan
Full list of author information is available at the end of the article

agents of ARI, and these viruses are also associated with the common cold, bronchiolitis, and pneumonia [14]. However, the relationships between these viruses and virus-induced wheezing are not exactly known.

Genetic analyses including sequence and phylogenetic analyses of various viruses enable detailed genetic characterization of these agents. With the use of these methods, detailed molecular epidemiological studies have been reported, even in non-culturable viruses such as HRV species C (HRV-C) or HBoV [15,16]. However, molecular epidemiology of various respiratory viruses with regard to virus-induced asthma is not exactly known. From these backgrounds, we detected various respiratory viruses and performed a molecular epidemiological study of them in Japanese children with acute wheezing illness.

Methods

Subjects

One hundred fifteen wheezy Japanese children were enrolled in the present study. A summary of patient data is shown in Table 1. All patients visited the National Hospital Organization Yokohama Medical Center from November 2007 to March 2009. Of these patients, 39 had a history of wheezing, while the other 76 patients had no such history. In addition, 66 patients had viral bronchitis and/or bronchiolitis at consultation. These patients were treated with infusion, oxygen, and β2-agonist or epinephrine nebulization. Informed consent was obtained from the parents of all subjects for the donation of the nasopharyngeal swabs used in this study.

DNA/RNA extraction, PCR, and sequencing

For viral DNA/RNA extraction, RT-PCR, and sequence analysis, nasopharyngeal swab samples were centrifuged at 3000 × g at 4°C for 15 min, and the supernatants were used for RT-PCR and sequence analysis as described previously [17]. Viral nucleic acid was extracted from the samples using the High Pure Viral Nucleic Acid Kit (Roche Diagnostics, Mannheim, Germany). The reverse transcription reaction mixture was incubated with random hexamers at 42°C for 90 min, followed by incubation at 99°C for 5 min, and then amplification by thermal cycling. The PCR procedures for amplification of various viral genes including RSV [18], HRV [19,20], HMPV [21], HPIV [22], EV [19,20], InfV [23], AdV [24], and HBoV [25] were conducted as previously described. The primers for PCR are shown in Table 2. To avoid carry over and cross-contamination in PCR, the extraction of viral RNA/DNA was conducted in a room physically separate from that used for performing PCR. Furthermore, positive and negative controls were included in all PCR assays. PCR products were determined by electrophoresis on 3% agarose gel. Purification of DNA fragments and nucleotide sequence determination procedures were performed as described previously [17].

Phylogenetic analysis and calculation of pairwise distances

We performed homology and phylogenetic analysis of the *G* gene of RSV, and the *VP4/VP2* coding region of HRV, because these viruses were the most commonly detected strains. The nucleotide positions of the nucleotide positions of the *G* gene of RSV were 673-912 (240 bp, for subgroup A) or 670-963 (294 bp, for subgroup B), and the *VP4/VP2* coding region of HRV were 623-1012 (390 bp). We used the CLUSTAL W program on the DNA Data Bank of Japan (DDBJ) homepage http://clustalw.ddbj.nig.ac.jp/top-j.html and TreeExplorer (Version 2.12) http://evolgen.biol.metro-u.ac.jp/TE/. Evolutionary distances were estimated using Kimura's two-parameter method, and phylogenetic trees were constructed using the neighbor-joining (NJ) method [26]. The reliability of the tree was estimated using 1000 bootstrap replications. We selected the reference strains as previously described to construct the phylogenetic trees of RSV and HRV

Table 1 Subject data in this study

No. of patients	Sex(M/F)	Age (months)	History of wheezing and/or asthma	No. of patients	Sex(M/F)	Age (months)	No. of inpatients and outpatients		No. of cases of bronchitis and/or bronchiolitis	Age (months)	Hospitalization (days)
115	70/45	20.8 ± 25.7	No	76	44/32	16.9 ± 23.9	inpatients	55*	46*	13.5 ± 21.0	7.1 ± 2.5
							outpatients	21	7	25.9 ± 28.9	
			Yes	39	26/13	28.5 ± 27.5*	inpatients	16	8	18.1 ± 17.0	7.1 ± 1.2
							outpatients	23	5	35.7 ± 31.3	

Data are expressed as mean ± SD

M/F: male/female

*$p < 0.05$

Table 2 Primers for PCR used in this study

Virus	Primer	Sequence	Reference no.
RSV	ABG490	5'-ATGATTWYCAYTTTGAAGTGTTC-3'	[22]
	F164	5'-GTTATGACACTGGTATACCAA CC-3'	
	AG655	5'-GATCYCAAACCTCAAACCAC-3'	[23]
	BG517	5'-TTYGTTCCCTGTAGTATATGT G-3'	
HRV	EVP4	5'-CTACTTTGGTGTCCGTGTT-3'	[24]
	OL68-1	5'-GGTAAYTTCCACCACCANCC-3'	[25]
HMPV	hMPV-1f	5'-CTTTGGACTTAATGACAGATG-3'	[26]
	hMPV-1r	5'-GTCTTCCTGTGCTAACTTTG-3'	
	hMPV-2f	5'-CATGCCGACCTCTGCAGGAC-3'	[27]
	hMPV-2r	5'-ATGTTGCAYTCYYTTGATTG-3'	
HPIV	PIS1+	5'-CCGGTAATTTCTCATACCTAT G-3'	[28]
	PIS1-	5'-CTTTGGAGCGGAGTTGTTAAG-3'	
	PIS2+	5'-CCATTTACCTAAGTGATGGAAT-3'	
	PIS2-	5'-GCCCTGTTGTATTTGGAAGAGA-3'	
	PIS3+	5'-ACTCCCAAAGTTGATGAAAGAT-3'	
	PIS3-	5'-TAAATCTTGTTGTTGAGATTG-3'	
InfV A	M30F2/08	5'-ATGAGYCTTYTAACCGAGGTCGAAACG-3'	[29]
	M264R3/08	5'-TGGACAAANCGTCTACGCTGCAG-3'	
InfV B	BHA1F1	5'-AATATCCACAAAATGAAG GCAATA-3'	[29]
	BHAR1166	5'-ATCATTCCTTCCCATCCTCCTTCT-3'	
AdV	AdnU-S'2	5'-TTCCCCATGGCNCACAAYAC-3'	[30]
	AdnU-A2	5'-TGCCKRCTCATRGGCTGRAAGTT-3'	
HBoV	188F	5'-GACCTCTGTAAGTACTATTAC-3'	[31]
	542R	5'-CTCTGTGTTGACTGAATACAG-3'	

RSV: respiratory syncytial virus; HRV: human rhinovirus; HMPV: human metapneumovirus; HPIV: human parainfluenza virus; InfV A: influenza virus subtype A; Inf B: influenza virus subtype B; AdV: adenovirus; HBoV: human bocavirus

[17,27]. Moreover, we calculated subgroup or species frequency distributions using pairwise genetic distances for each strain, as previously described [17].

Statistical analysis

Data were analyzed using SPSS software (SPSS for Windows, Version 10.0). All data are expressed as mean ± SD. We performed bivariate analyses using Pearson χ^2 and Fisher exact tests to compare the prevalence of respiratory viruses and other variables between the study groups. The Student's *t*-test was used to compare mean age in the study group. Statistical significance was set at the level of $p< 0.05$.

Ethics approval

All samples were collected after written informed consent was obtained from the subjects' parents. The study protocol was approved by the Ethics Committee on Human Research of National Hospital Organization Yokohama Medical Center.

Results

Viruses detected in the present subjects

We genetically detected RSV, HRV, HMPV, HPIV, EV, InfV, AdV, and HBoV in samples from 115 Japanese children with acute wheezing (Table 3). RSV alone was detected in 47 patients (40.9%). Among these, subgroups A (RSV-A) and B (RSV-B) were found in 27 and 20 patients, respectively. HRV alone was detected in 36 patients (31.3%), and among these, HRV species A (HRV-A), B (HRV-B), and C (HRV-C) were found in 17, 2, and 17 patients, respectively. Both RSV and HRV were detected in 14 patients (12.2%). Among these, combinations of RSV-A + HRV-A, RSV-A + HRV-B, and RSV-A + HRV-C were found in 5, 1, and 1 patient, respectively. In addition, RSV-B + HRV-A, RSV-B + HRV-B, and RSV-B + HRV-C were found in 2, 1, and 4 patients, respectively. HMPV alone and HPIV alone were detected in 1 patient each, respectively. Finally, no viral genes for RSV, HRV, HMPV, HPIV, EV, InfV, AdV, and HBoV were detected in 16 patients (13.9%). From these data, RSV was revealed to be the dominant species detected in patients with no history of wheezing and/or asthma (38 patients vs. 9 patients, $p< 0.05$), while HRV was dominant in those with a history of wheezing and/or asthma (12 patients vs. 24 patients, $p< 0.05$). These results suggested that RSV and HRV were the major causative agents of acute wheezing in the present study. Moreover, both RSV and HRV were detected in over 10% of patients with acute wheezing.

Seasonal variations of detected viruses

To address relationships between seasonal variations of respiratory viruses and acute wheezing, we showed detected viruses during investigation period as Figure 1. Prevalence of RSV was found from autumn to winter, while prevalence of HRV was found in all season. In addition, both viruses were detected from autumn to winter.

Homology, phylogenetic analysis, and pairwise distances of RSV and HRV

We performed phylogenetic and homology analysis, and calculated the pairwise distances of RSV and HRV in the present cases. The phylogenetic tree based on *G* gene of RSV, and the *VP4/VP2* coding region of HRV are shown in Figure 2 and 3. The homology and pairwise distances are shown in Table 4. First, the RSV was classified into two subgroups, A and B. In addition, strains belonging to both subgroups were subdivided into three genotypes (GA2, GA5, and BA, Figure 2). HRV was classified into three species: HRV-A, -B, and -C. Strains belonging to these species were subdivided into many clusters in the phylogenetic tree (Figure 3). The homology of RSV-A was relatively high (over 80%), while it was quite low for other viruses and all species

Table 3 Subtypes or species of detected viruses

	No history of wheezing and/or asthma		Having history of wheezing and/or asthma	
Virus	No. of strains	Strain name	No. of strains	Strain name
RSV-A	25	RSV/YOK/07/14(AB551036), RSV/YOK/07/22(AB551037), RSV/YOK/07/26(AB551038), RSV/YOK/07/43(AB551039), RSV/YOK/07/51(AB551040), RSV/YOK/07/52(AB551041), RSV/YOK/07/53(AB551042), RSV/YOK/07/66(AB551044), RSV/YOK/08/79(AB551046), RSV/YOK/08/83(AB551047), RSV/YOK/08/113(AB551049), RSV/YOK/08/122(AB551053), RSV/YOK/08/123(AB551054), RSV/YOK/08/125(AB551056), RSV/YOK/08/127(AB551057), RSV/YOK/08/128(AB551058), RSV/YOK/08/130(AB551059), RSV/YOK/08/133(AB551060), RSV/YOK/08/134(AB551061), RSV/YOK/08/141(AB551065), RSV/YOK/08/142(AB551066), RSV/YOK/08/146(AB551069), RSV/YOK/08/148(AB551071), RSV/YOK/08/150(AB551072), RSV/YOK/09/162(AB551075)	2	RSV/YOK/08/73(AB551045), RSV/YOK/08/111(AB551048)
RSV-B	13	RSV/YOK/07/13(AB551078), RSV/YOK/07/16(AB551079), RSV/YOK/07/17(AB551080), RSV/YOK/07/21(AB551081), RSV/YOK/07/32(AB551083), RSV/YOK/07/33(AB551084), RSV/YOK/07/34(AB551085), RSV/YOK/07/38(AB551086), RSV/YOK/07/50(AB551092), RSV/YOK/07/56(AB551093), RSV/YOK/07/60(AB551095), RSV/YOK/07/62(AB551096), RSV/YOK/07/64(AB551097)	7	RSV/YOK/07/4(AB551076), RSV/YOK/07/59(AB551094), RSV/YOK/08/74(AB551102), RSV/YOK/08/80(AB551104), RSV/YOK/08/82(AB551105), RSV/YOK/08/84(AB551106), RSV/YOK/08/88(AB551107)
Subtotal	38		9	
HRV-A	5	HRV/YOK/07/7(AB550346), HRV/YOK/07/61(AB550365), HRV/YOK/08/107(AB550377), HRV/YOK/08/110(AB550379), HRV/YOK/08/112(AB550380)	12	HRV/YOK/07/11(AB550348), HRV/YOK/07/15(AB550350), HRV/YOK/07/19(AB550352), HRV/YOK/07/24(AB550355), HRV/YOK/07/25(AB550356), HRV/YOK/07/36(AB550358), HRV/YOK/08/103(AB550374), HRV/YOK/08/131(AB550389), HRV/YOK/08/153(AB550396), HRV/YOK/08/167(AB550402), HRV/YOK/08/169(AB550403), HRV/YOK/08/171(AB550404)
HRV-B	1	HRV/YOK/08/129(AB550389)	1	HRV/YOK/08/154(AB550397)
HRV-C	6	HRV/YOK/07/5(AB550345), HRV/YOK/07/20(AB550356), HRV/YOK/07/41(AB550368), HRV/YOK/08/100(AB550379), HRV/YOK/09/163(AB550400), HRV/YOK/09/164(AB550401)	11	HRV/YOK/07/2(AB550343), HRV/YOK/07/10(AB550347), HRV/YOK/07/12(AB550349), HRV/YOK/07/18(AB550351), HRV/YOK/07/23(AB550353), HRV/YOK/07/55(AB550371), HRV/YOK/08/86(AB550377), HRV/YOK/08/106(AB550382), HRV/YOK/08/120(AB550386), HRV/YOK/08/126(AB550388), HRV/YOK/08/159(AB550398)
Subtotal	12		24	
RSV-A +HRV-A	2	RSV/YOK/08/116(AB551050) + HRV/YOK/08/116(AB550381), RSV/YOK/08/145(AB551068) + HRV/YOK/08/145(AB550392)	3	RSV/YOK/07/1(AB551033) + HRV/YOK/07/1(AB550342), RSV/YOK/08/117(AB551051) + HRV/YOK/08/117(AB550382), RSV/YOK/08/119(AB551052) + HRV/YOK/08/119(AB550384)
RSV-A +HRV-B		ND	1	RSV/YOK/08/140(AB551064) + HRV/YOK/08/140(AB550392)
RSV-A +HRV-C		ND	1	RSV/YOK/07/3(AB551034) + HRV/YOK/07/3(AB550344)
RSV-B +HRV-A	2	RSV/YOK/07/42(AB551087) + HRV/YOK/07/42(AB550361), RSV/YOK/07/47(AB551090) + HRV/YOK/07/47(AB550363)		ND
RSV-B +HRV-B	1	RSV/YOK/08/118(AB551108) + HRV/YOK/08/118(AB550363)		ND
RSV-B +HRV-C	4	RSV/YOK/07/28(AB551082) + HRV/YOK/07/28(AB550365), RSV/YOK/07/45(AB551088) + HRV/YOK/07/45(AB550405), RSV/YOK/07/46(AB551089) + HRV/YOK/07/46(AB550370), RSV/YOK/07/67(AB551099) + HRV/YOK/07/67(AB550375)		ND
Subtotal	9		5	
HMPV-B2	1	HMPV/YOK/07/44(AB565438)		ND
HPIV-1		ND	1	HPIV/YOK/08/115(AB565748)
Total	60		39	

RSV-A, Respiratory syncytial virus subgroup A;RSV-B, Respiratory syncytial virus subgroup B; HRV-A, Human rhinovirus species A; HRV-B, Human rhinovirus species B; HRV-C, Human rhinovirus species C; HMPV, Human metapneumovirus; HPIV-1, Human parainfluenza virus type 1; ND, Not detected

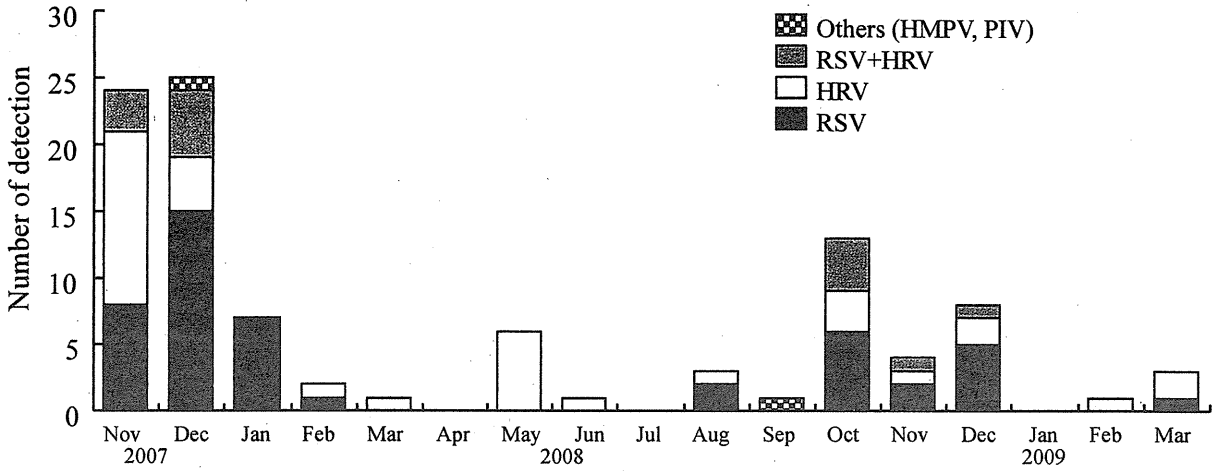

Figure 1 Seasonal variations of respiratory viruses detected in this study.

of HRV (over 30% divergence). Notably, the genetic diversity of HRV-C was wide (52 to 100%). In addition, the pairwise distances of HRV-A and HRV-C strains are high (over 0.2), while those for RSV-A and RSV-B strains are low. Based on these results it is suggested that acute wheezing-associated HRV has wide genetic diversity.

Discussion

We detected and genetically analyzed major ARI viruses including RSV, HRV, HMPV, and HPIV in samples from 115 Japanese children with acute wheezing during a 17-month period (November 2007 and March 2009). These viruses were detected in over 80% of the patients. The dominant viruses were RSV and HRV, and both were detected in over 10% of the patients. In addition, these viruses were confirmed as belonging to various subgroups, genotypes, or species. All three species of HRV detected showed wide genetic diversity (more than 30% divergence). Interestingly, RSV was the dominant species detected in patients with no history of wheezing and/or asthma, while HRV was dominant in patients with a history of wheezing and/or asthma. The results suggested that RSV and HRV were major ARI viruses regarding virus-induced acute wheezing in the present study.

It is suggested that various respiratory viruses such as RSV, HRV, HMPV, HPIV, EV, InfV, AdV, and HBoV are detected in patients with lower respiratory tract infections including bronchiolitis and pneumonia [6,7]. These viruses are also detected in cases of acute wheezing [6,7]. Thus, they may be associated with both lower respiratory tract infection and acute wheezing in children [6,7]. At present, this disease status is recognized by physician and pediatrician as virus-induced asthma [28,29]. It may be important to address the genetic properties of ARI viruses associated with these diseases. However, few studies have been conducted into the genetic analysis of these viruses in acute wheezing. To better understand the relationships between viral properties and acute wheezing, it may be important to genetically analyze ARI viruses detected in the wheezy children. We studied the molecular epidemiology of these respiratory viruses detected in Japanese children with acute wheezing. To the best of our knowledge, the present study is the first to report the detection of RSV, and HRV-A, -B, -C with different genetic characteristics in Japanese children with acute wheezing.

Many studies suggest that RSV is a major candidate as an inducer of acute wheezing [4,10,11] and it may infect all children under the age of 2 years [10,11]. Furthermore, some of these children may develop bronchiolitis and/or pneumonia with acute wheezing [10]. Sugai-Goto *et al.* demonstrated that genotypes and the major genes (*F, G,* and *N*) of RSV isolated from hospitalized children with bronchiolitis or bronchopneumonia accompanied by acute wheezing were not significantly different when compared with RSV strains detected from upper respiratory tract infections [27]. These viruses belong to subgroup A, genotype GA2 and subgroup B, genotype BA [27]. Furthermore, Nakamura *et al.* showed similar genetic data from various acute respiratory infections in Okinawa, Japan [30]. Our findings regarding the properties of *G* gene in the RSV strains detected were comparable with the above-mentioned reports. In contrast, it has been suggested that a specific genotype, GA3 type virus, might be associated with a significantly greater severity of illness [31]. Riccetto *et al.* demonstrated that the severity of illness of RSV infection in infants can be associated with other factors such as body weight and prematurity [32], and any

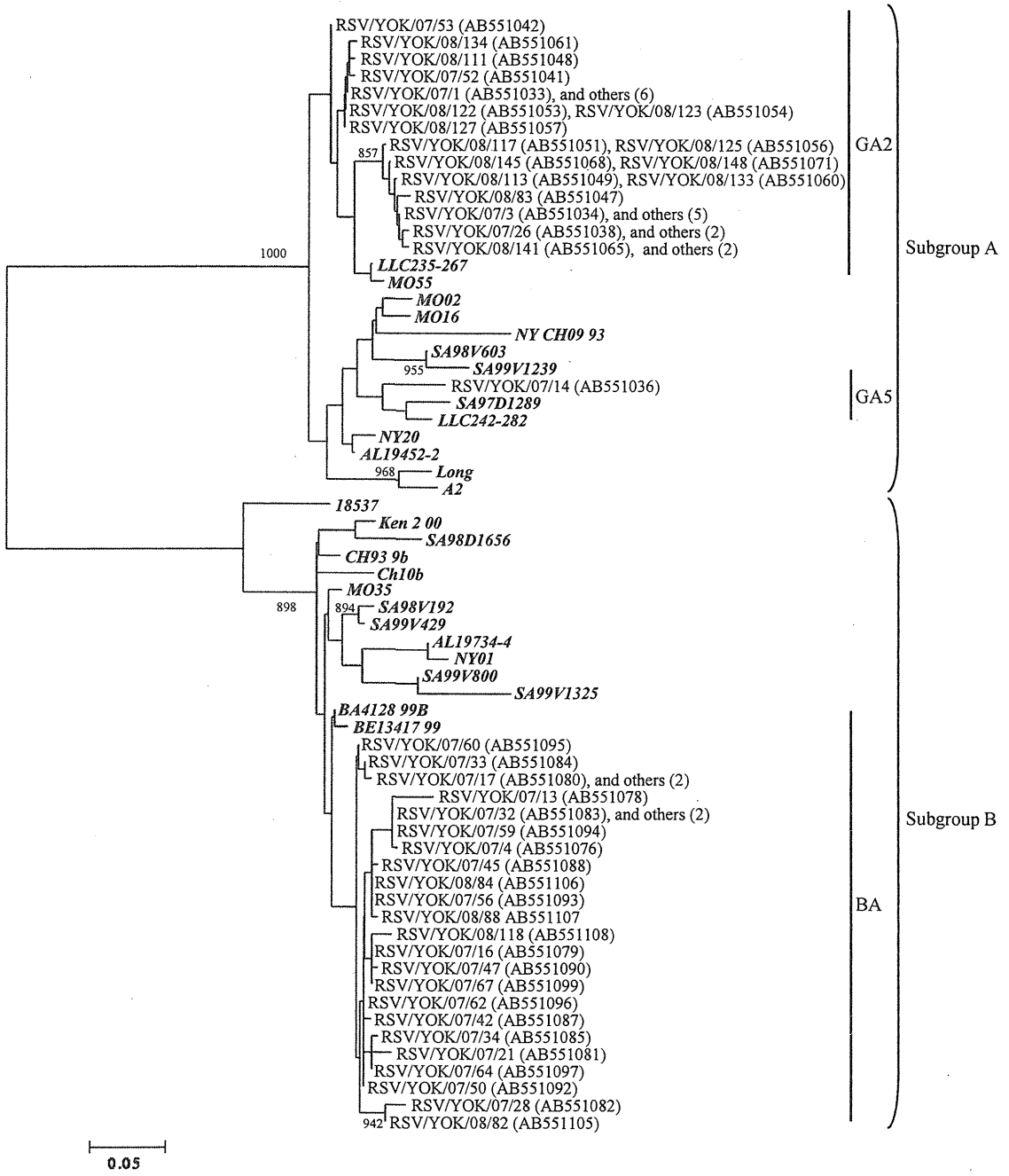

Figure 2 Phylogenetic analysis of RSV (G gene). Detailed procedures and conditions of the phylogenetic tree are shown in the text. Numbers in parentheses indicate numbers of strains detected in other patients. Reference strains are shown in bold type. Bars, 0.05 substitutions per nucleotide position. Only bootstrap values more than 85% are shown at branch points.

association between the viral properties and pathogenicity of RSV has yet to be elucidated. Another report suggested that host immunity such as TLR4 polymorphism is linked to symptomatic RSV [33]. Thus, both the antigenicity of the viruses and host immune conditions may play important roles in the pathophysiology of severe respiratory infections such as bronchiolitis, pneumonia, and virus-induced asthma [1,34].

For a long time, HRV was simply thought to be causative agents of the mild common cold [12]. In general,

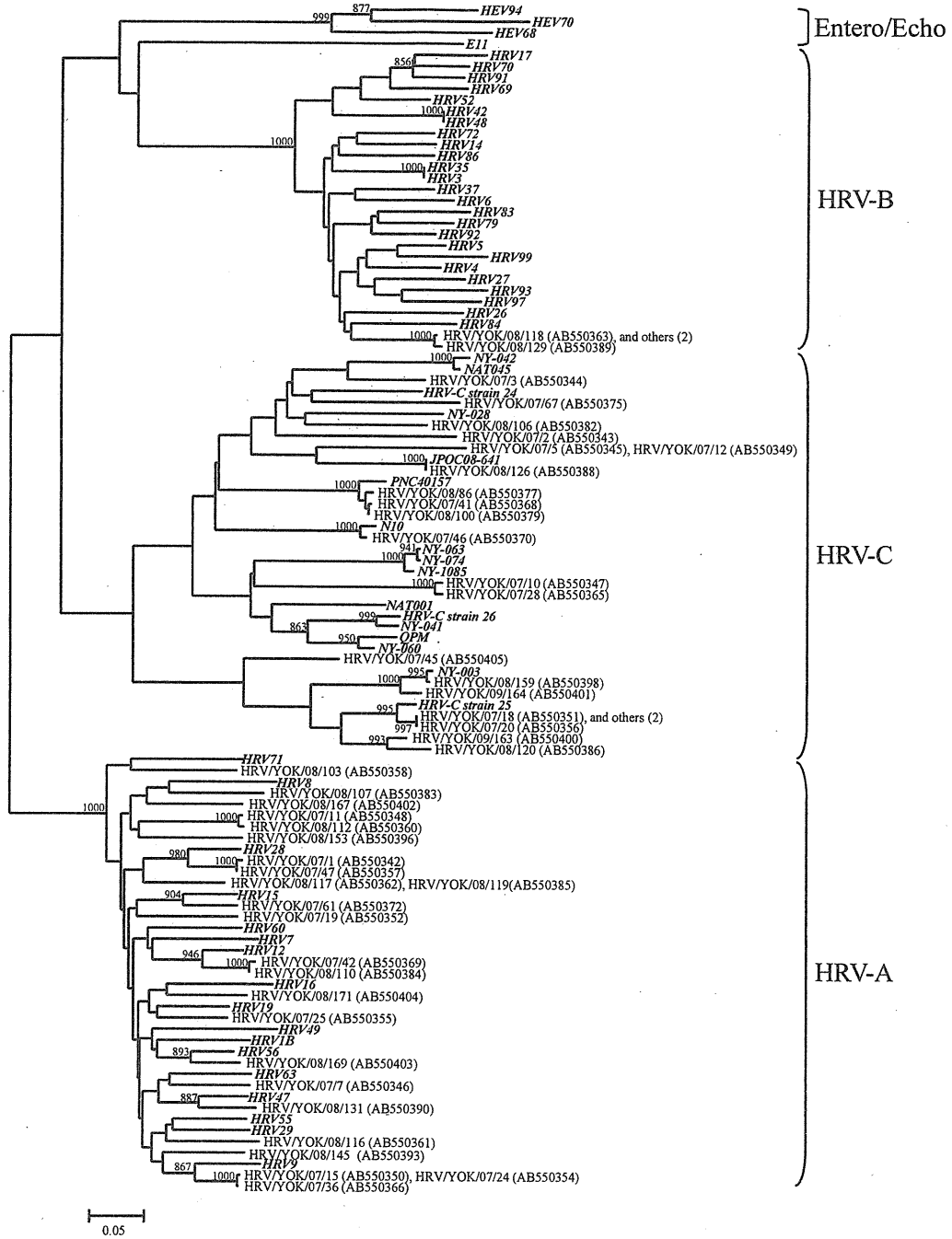

Figure 3 Phylogenetic analysis of HRV (*VP4/VP2* coding region). Detailed procedures and conditions of the phylogenetic tree are shown in the text. Numbers in parentheses indicate numbers of strains detected in other patients. Reference strains are shown in bold type. Bars, 0.05 substitutions per nucleotide position. Only bootstrap values more than 85% are shown at branch points.

this acknowledgement may not be incorrect in non-asthmatic people [35]. However, it is suggested that HRV induces wheezing and exacerbation of symptoms in most asthmatics [12]. However, the molecular epidemiology of each HRV species is not yet known, because HRV is relatively difficult to isolate and detect. Thus, non-culturable HRV-C was only recovered a few years ago. Very recently, Mizuta *et al.* demonstrated

Table 4 Pairwise distances and homology of RSV and HRV strains based on nucleotide sequences

	Homology (%)		Pairwise distance	
Virus	All strains*	Present strains**	All strains*	Present strains**
RSV-A	82.0 - 100	83.5 - 100	0.063 ± 0.043	0.035 ± 0.034
RSV-B	74.2 - 100	92.8 - 100	0.060 ± 0.040	0.029 ± 0.014
HRV-A	66.4 - 100	66.5 - 100	0.202 ± 0.031	0.200 ± 0.038
HRV-B	68.1 - 100	99.5 - 100	0.204 ± 0.039	0.002 ± 0.003
HRV-C	41.0 - 100	52.2 - 100	0.263 ± 0.069	0.254 ± 0.077

Data are expressed as mean ± SD

RSV-A, Respiratory syncytial virus subgroup A

RSV-B, Respiratory syncytial virus subgroup B

HRV-A, Human rhinovirus species A

HRV-B, Human rhinovirus species B

HRV-C, Human rhinovirus species C

* All strains: reference strains plus the strains detected in the present study.

** Present strains: viruses detected in the present study.

that HRV-A isolates showed wide genetic diversity, and some viruses belonging to specific clusters of the phylogenetic tree of HRV-A isolates might be associated with bronchiolitis [17]. In addition, a new study suggested that HRV-C has a stronger link to virus-induced asthma than HRV-A and -B strains [36]. However, our results did not reveal a similar tendency, although the reasons for this are unknown.

In this study, both RSV and HRV were detected from over 10% of patients with acute wheezing. Chung *et al.* demonstrated that both RSV and HRV were detected in 3.9% of Korean children with acute wheezing [37]. Thus, our data and that of other studies may be comparable, although the percentages of virus detection differ. We additionally compared the severity of clinical symptoms between dual virus-detected patients and those in whom RSV or HRV was detected alone. However, there were no significant findings. In addition, RSV, HRV, HMPV, HPIV, EV, InfV, AdV, and HBoV were not detected in over 10% of patients. Although we were unable to explain why, it might be that other respiratory viruses and bacteria were involved.

It should be noted that some respiratory viruses might be detected in healthy children [1,38-40]. As mentioned above, various species of HRV have been relatively frequently detected in healthy children (around 10-20%) [39,40], although RSV was detected less frequently in healthy and asymptomatic persons [40]. Thus, to better understand the etiology of these viruses, it may be important to determine the prevalence of these viruses in healthy children. A limitation of this study is that we did not examine such prevalence in healthy children and instead focused mainly on detailed molecular epidemiological analysis of various respiratory viruses detected in children with acute wheezing. Additional molecular epidemiological studies of viruses detected in wheezy and healthy children would be of value.

In the present study, HMPV and HPIV were detected in samples from the subjects, albeit rarely (each virus was detected in one of only two patients). It is suggested that HMPV and HPIV are also associated with bronchiolitis and bronchopneumonia [41]. However, it is not known how these viruses are linked to the induction of wheezing and exacerbation of asthma [42]. A previous study suggested that sputum from HPIV infection contains tryptase due to activation (degranulation) of mast cells, and this activation may strongly induce an asthmatic attack [43]. Thus, HPIV infection may induce asthmatic conditions [7]. Additional studies regarding the relationships between HPIV and HMPV infection and virus-induced asthma are warranted.

Conclusions

Our data suggested that both RSV and HRV with various genetic characteristics were associated with acute wheezing illness in Japanese children. In particular, HRV shows widely genetic diversity. Larger studies to examine the detailed genetic characteristics of the various respiratory viruses detected in wheezy and healthy children may be needed.

Abbreviations

RSV: respiratory syncytial virus; HRV: human rhinovirus; HMPV: human metapneumovirus; HPIV: human parainfluenza virus; EV: enterovirus; InfV: influenza viruses; AdV: adenoviruses; HBoV: human bocavirus; DDBJ: DNA Data Bank of Japan; NJ: neighbor-joining

Acknowledgements

We thank Drs. Hirotaka Motoi and Yoshinori Kobayashi for constructive discussion. This work was supported by a Grant-in-Aid from the Japan Society for Promotion of Science and for Research on Emerging and Re-emerging Infectious Diseases from the Ministry of Health, Labour and Welfare.

Author details

[1]Department of Pediatrics, National Hospital Organization Yokohama Medical Center, 3-60-2 Harajuku, Totsuka-ku, Yokohama, Kanagawa 245-8575, Japan. [2]Department of Pediatrics, Yokohama City University Graduate School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama, Kanagawa 236-0004, Japan. [3]Department of Health Science, Gunma Prefectural Institute of Public Health and Environmental Sciences, 378 Kamioki-machi, Maebashi-shi, Gunma 371-0052, Japan. [4]Department of Microbiology, Tochigi Prefectural Institute of Public Health, 2154-13 Shimo-okamoto, Utsunomiya-shi, Tochigi 329-1196, Japan. [5]Department of Molecular Biodefence Research, Yokohama City University Graduate School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama, Kanagawa 236-0004, Japan. [6]Division of Molecular Cell Immunology and Allergology, Advanced Medical Research Center, Nihon University, Graduate School of Medical Science, 30-1 Oyaguchi-kamimachi, Itabashi-ku, Tokyo 173-8610, Japan. [7]Department of Microbiology, Yamagata Prefectural Institute of Public Health, 1-6-6 Toka-machi, Yamagata-shi, Yamagata 990-0031, Japan. [8]Department of Health and Nutrition, Yamagata Prefectural Yonezawa Women's Junior College, 6-15-1Tori-machi, Yonezawa-