Received: 7 October 2020 | Revised: 12 March 2021 | Accepted: 6 April 2021

DOI: 10.1002/jmv.27004

RESEARCH ARTICLE

JOURNAL OF MEDICAL VIROLOGY WILEY

Epidemiology of acute respiratory viral infections in children in Vientiane, Lao People's Democratic Republic

Chantal J. Snoeck[1] | Konstantin Evdokimov[1] | Kinnaly Xaydalasouk[2] | Sodaly Mongkhoune[3] | Aurélie Sausy[1] | Keoudomphone Vilivong[2,4] | Maude Pauly[1] | Judith M. Hübschen[1] | Somxay Billamay[3] | Claude P. Muller[1] | Antony P. Black[2]

[1]Department of Infection and Immunity, Luxembourg Institute of Health, Esch-sur-Alzette, Luxembourg

[2]Institut Pasteur du Laos, Vientiane, Lao PDR

[3]Children Hospital, Vientiane, Lao PDR

[4]Wellcome Trust Research Unit, Lao-Oxford-Mahosot Hospital, Vientiane, Lao PDR

Correspondence
Chantal J. Snoeck, Department of Infection and Immunity, Luxembourg Institute of Health, 29 rue Henri Koch, L-4354 Esch-sur-Alzette, Luxembourg.
Email: chantal.snoeck@lih.lu

Funding information
Luxembourg Institute of Health; Ministry of Foreign and European Affairs, Luxembourg, Grant/Award Number: PaReCIDS II

Abstract

Respiratory infections are one of the most frequent reasons for medical consultations in children. In low resource settings such as in Lao People's Democratic Republic, knowledge gaps and the dearth of laboratory capacity to support differential diagnosis may contribute to antibiotic overuse. We studied the etiology, temporal trends, and genetic diversity of viral respiratory infections in children to provide evidence for prevention and treatment guidelines. From September 2014 to October 2015, throat swabs and nasopharyngeal aspirates from 445 children under 10 years old with symptoms of acute respiratory infection were collected at the Children Hospital in Vientiane. Rapid antigen tests were performed for influenza A and B and respiratory syncytial virus. Real-time reverse-transcription polymerase chain reactions (RT-PCRs) were performed to detect 16 viruses. Influenza infections were detected with a higher sensitivity using PCR than with the rapid antigen test. By RT-PCR screening, at least one pathogen could be identified for 71.7% of cases. Human rhinoviruses were most frequently detected (29.9%), followed by influenza A and B viruses combined (15.9%). We identify and discuss the seasonality of some of the infections. Altogether these data provide a detailed characterization of respiratory pathogens in Lao children and we provide recommendations for vaccination and further studies.

KEYWORDS

acute respiratory infections, children, human metapneumovirus, influenza virus, Lao PDR, respiratory syncytial virus

This paper is dedicated to the memory of Dr. Somxay Billamay, who passed away in January 2021.

1 | INTRODUCTION

In children, respiratory tract infections are one of the most common reasons for seeking medical care.[1] In Lao People's Democratic Republic (PDR), consultations for influenza-like illness (ILI) represented 10% of outpatient consultations in Vientiane between 2008 and 2010.[2] The incidence of ILI was estimated at 10.7 episodes per 100 person years in the Vientiane metropolitan area between 2015 and 2016, with the highest rates in children below 15 years old.[3] The etiology of respiratory tract infections is very diverse. A high proportion of viral infections present with mild symptoms,[4,5] which may worsen due to bacteria superinfections.[6] Measures to mitigate bacteria coinfections or gaps in differential diagnosis often lead to empirical antibiotic treatment. Antibiotic prescription levels in primary care settings in low and middle-income countries are often high,[7] and are influenced by staff training level. However, innate and adaptive immune responses to pathogenic microorganisms are influenced by commensal bacteria, which are present in site-specific communities on mucosal surfaces.[8] Resident non-pathogenic bacteria of the airways augment the immunity against viral infections.[9] Therefore, empirical antibiotic treatment of respiratory infections without a laboratory confirmation may disrupt the balance necessary for effective virus clearance. In addition, unnecessary antibiotic administration increases the risk of emergence and spread of antibiotic resistance.

In Lao PDR, knowledge gaps in respiratory tract infection diagnosis and management exist. In a previous study, nearly 30% of the medical doctors questioned thought that unnecessary antibiotic treatment was harmless.[10] In addition, weak laboratory capacity to substantiate differential diagnosis can lead to unnecessary antibiotic prescription, often driven by patient demand or antibiotic availability rather than necessity.[10] A surge of patients with ILI was reported between May and July 2008 in Vientiane but this did not coincide with increased transmission of influenza cases.[11] Previous studies in the country were confined to a limited number of pathogens, mainly influenza viruses, with almost half of all patients left without etiological diagnosis.[2,11,12] This, combined with the age-specific pathogen susceptibility, warrants a broadening of respiratory surveillance programs. This study therefore aimed at investigating the etiology of respiratory infections in children, a population with higher susceptibility and disease severity. The study aimed at including a larger panel of viral agents than previous studies, to provide better evidence on disease etiology to local medical doctors. It aimed at investigating seasonal trends of virus circulation and to genetically characterize influenza A virus, respiratory syncytial virus, and human metapneumovirus, often responsible for more severe disease outcomes in young children.

2 | MATERIALS AND METHODS

2.1 | Ethics approval and consent to participate

The study was approved by the Lao National Ethics Committee for Health Research (No. 027/NIOPH/NECHR). Clinical samples and data were collected after obtaining informed consent from the patient's parents or legal guardians.

2.2 | Inclusion criteria, sample and data collection

The inclusion criteria were: age below 10-year old, presenting to the Children Hospital in Vientiane with symptoms of acute respiratory infection; informed consent obtained. Throat swabs and nasopharyngeal aspirates were collected by trained hospital personnel. A questionnaire capturing demographic and clinical data was administered by health care personnel.

2.3 | Virus detection and characterization

Rapid antigen (Ag) tests for influenza A/B/A (H1N1) and respiratory syncytial virus (RSV) (SD Bioline) were performed on the nasopharyngeal aspirates on the day of collection according to manufacturer's instructions. The remaining material was transported to the Institut Pasteur du Laos (IPL) where nucleic acid purification with TRIzol LS (Life Technologies) and Pure Link Viral RNA/DNA Mini Kits (Life Technologies) was performed. RNA and DNA were eluted in RNAse free water and stored at −80°C and −20°C, respectively.

Influenza A virus detection was performed on throat swabs by real-time reverse-transcription polymerase chain reaction (RT-PCR) with MP-39-67For, MP-183-153Rev primers, and MP-96-75ProbeAs probe[13] and QuantiTect Probe RT-PCR Kit (Qiagen). Detection of influenza B and C virus, RSV, human metapneumovirus (hMPV), parainfluenzaviruses 1-4 (PIV1-4), human coronaviruses 229E, OC43, HKU1 and NL63 (hCoV-229E, -OC43, -HKU1, -NL63) and human rhinoviruses (hRV) was performed separately on both throat swabs and nasopharyngeal aspirates in four conventional multiplex PCR assays using Qiagen OneStep RT-PCR Kit (Qiagen).[14] Human adenovirus (hAdV) and human Bocavirus (hBoV) were detected in a pool of equal volume of DNA purified from both types of samples by a fifth multiplex PCR using TaqMan Universal PCR Master Mix (Life Technologies). Influenza A virus subtyping was performed by two real-time RT-PCRs detecting pandemic H1N1 strains (A/H1N1pdm) or seasonal H3N2 strains (A/H3N2).[13] RSV positive samples were identified as RSV-A or RSV-B by a duplex real-time RT-PCR with QuantiTect Probe RT-PCR Kit.[15]

Complete glycoprotein (G) sequences of RSV strains and partial fusion (F) and glycoprotein (G) sequences of hMPV were amplified using previously published[16,17] and newly designed primer pairs. PCR product purification and sequencing were performed as described before.[18] The nucleotide substitution model that best fitted the data was determined with MEGA6.[19] Genetic distances and phylogenetic analyses were then calculated with the best model using the Neighbor-joining method and 500 bootstrap replicates in MEGA6.

2.4 | Data analyses

Statistical analyses were performed using SigmaPlot version 12.5. McNemar's test with Yates correction for continuity was used for comparing detection rates between swab types or between RT-PCR and rapid tests. Mann–Whitney rank-sum tests were used to assess the effect of age on overall positivity rate (≥1 virus detected), on the detection of single or mixed infections, on the positivity rate for each virus, on antibiotic prescription, and to assess the effect of delay between onset of symptoms and sampling on the outcome of rapid tests. A one-tailed *t* test was applied to assess the effect of influenza C_t values on rapid test outcomes. Odds ratios were calculated to describe the risk associated with positivity for each viral species and hospital admission. Odds ratios were also calculated to evaluate antibiotic prescription with regard to in- or outpatient treatment.

Based on the sample collection date, cases were classified as occurring during the rainy or dry seasons. In Lao PDR, the rainy season, typically taking place between mid-October and mid-May of the following year, is marked by frequent and heavy rain with high humidity. Rainy seasons alternate with dry seasons characterized by low humidity and high temperatures that occurs from mid-May to mid-October.[20] Z-scores were calculated to assess the seasonality of virus occurrence in the dry or rainy season.

3 | RESULTS

3.1 | Demographic data

From September 2014 to August 2015, 445 children (median age 18.6 months; Table 1) were recruited at the Children's Hospital in Vientiane. Patients presented with fever ($n = 390$) and/or cough ($n = 418$) and/or nasal congestion ($n = 403$; Table 1). The majority (80.9%) were outpatients while 19.1% (85 of 445) were admitted to the hospital. Throat swabs and nasopharyngeal aspirates were collected from all patients ($n = 445$).

3.2 | Prevalence of respiratory viruses by PCR

Overall, at least one virus was detected by RT-PCR in 71.7% (319 of 445; Table 1) of the patients when combining results from both types of swabs. hRV was the most frequently detected pathogen (133 of 445, 29.9%) followed by influenza A virus (54 of 445, 12.1%; Figure 1). All other viruses were detected at lower levels (2.7%–8.1%) and PIV2, PIV4, and the 4 hCoVs were detected only sporadically; in less than 2% of the patients (Figure 1). Although detection rates were the same or higher in nasopharyngeal aspirates compared to throat swabs for most pathogens (10 of 13), a statistically significant difference was only observed for RSV ($p = .039$; Figure 1B).

Mixed infections were detected in 13.5% (60 of 445; Table 1) of the patients, with a majority of dual viral infections (57 of 60, 95.0%) compared to triple viral co-infections (3 of 60, 5.0%). Nineteen pathogen combinations were found, most combinations (13 of 19, 64.8%) being observed only in 1 or 2 patients. hRV was involved in 75.0% (45 of 60) of the mixed infections and most frequent combinations involved hRV together with hAdV (15 of 60, 25.0%) or together with influenza C virus (10 of 60, 16.7%). No statistically significant difference between the age of children with single or mixed infection was observed ($p = .542$).

3.3 | Comparison of rapid antigen detection and PCR detection methods

Detection rates of influenza A and B viruses by rapid tests (38 of 432, 8.8%, 13 samples were not tested by rapid tests) were significantly lower than cumulative detection by the two PCRs (71 of 432, 16.4%; $p < .001$). These results suggest a high rate of false negatives by rapid antigen tests. For the patients with influenza A and B infections confirmed by PCR, the delay between the reported date of symptom onset and sampling did not affect the outcome of detection by rapid test ($p = .348$). For influenza A,

TABLE 1 Demographic, clinical, and virus detection rate data of the 445 patients enrolled

Variable	No. of patients (%)
Gender	
Female	190 (42.7)
Male	255 (57.3)
Age group	
0–6 months	55 (12.4)
7–12 months	94 (21.1)
13–24 months	130 (29.2)
25–60 months	131 (29.4)
5–10 years	35 (7.9)
Patient admission	
Outpatient	360 (80.9)
Inpatient	85 (19.1)
Symptoms	
Fever	390 (87.6)
Cough	418 (93.9)
Nasal congestion	403 (90.6)
Detection of viral infections[a]	
Positive for at least 1 virus	319 (71.7)
Single viral infection	259 (58.2)
Mixed viral infections[b]	60 (13.5)

[a]Positivity by real-time reverse-transcription polymerase chain reaction (RT-PCR) results from throat swabs and nasopharyngeal aspirates combined.

[b]Includes the number of cases where two or three viruses were detected.

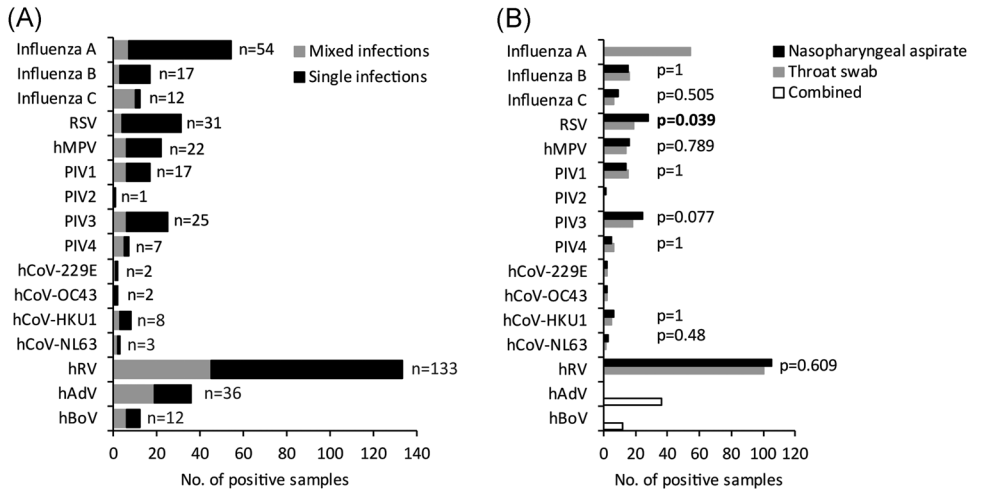

FIGURE 1 (A) Number of patients tested positive by RT-PCR for each virus involved in single infections (black) or mixed infections (gray; include cases of both dual and triple infections. (B) Number of positive samples according to the sample type, that is, nasopharyngeal aspirates or throat swabs. hAdV and hBoV were detected in a pool of equal volume of DNA purified from both types of samples, referred to as "combined." When applicable, *p* value of McNemar's test comparing detection rates in both types of samples is provided. hCoV, human coronavirus; hMPV, human metapneumovirus; hRV, human rhinovirus; PIV, parainfluenza virus; RSV, respiratory syncytial virus; RT-PCR, real-time reverse-transcription polymerase chain reaction

samples with higher C_t values were less likely to be positive by rapid test ($p = .026$). No statistically significant difference was observed when comparing RSV detection by rapid test (37 of 436, 10.8%) or conventional PCR (28 of 436, 6.4%; $p = .814$). Nine RSV rapid test positive specimens were PCR negative, which may indicate either a lack in sensitivity of the conventional PCR and/or false positives by rapid tests.

3.4 | Age distribution, disease severity, and antibiotic treatment

The overall positivity rate (≥1 virus detected) and presence of mixed infections were not associated with age ($p = .556$ and $p = .542$, respectively). Patients with influenza A ($p = .017$), influenza B ($p = .004$) or hAdV ($p = .026$) infections were significantly

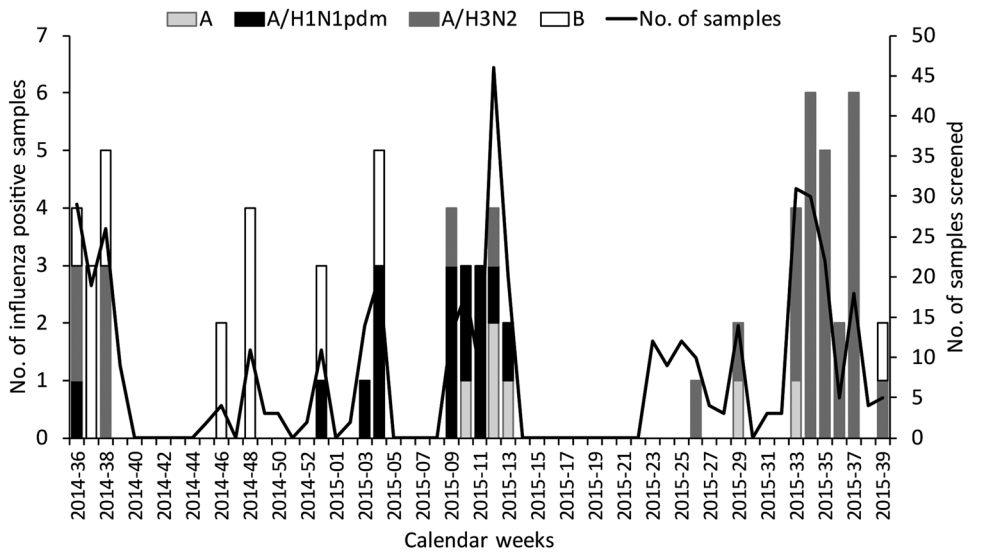

FIGURE 2 Seasonal incidence of influenza A and B viruses in Lao PDR (color-coded bars) and number of samples tested (line) by calendar weeks

older. Patients with RSV ($p = .010$) or hRV ($p = .046$) infections were significantly younger.

Only RSV infection was associated with an increased risk of hospital admission (OR = 2.527, $p = .030$). Antibiotic prescription was reported for 23.1% (103 of 445) children. Inpatients were more likely to receive antibiotics than outpatients (30 of 85, 35.3% vs. 73 of 360, 20.3%; odd ratio = 2.144, $p = .005$), but there was no relationship between age and antibiotic prescription ($p = .439$).

3.5 | Virus typing and seasonal trends

Influenza A was detected in 12.1% of patients (54 of 445) and the strains were characterized as A/H1N1 pdm (16 of 54, 29.6%) and A/H3N2 (32 of 54, 59.3%). Influenza B was detected in 3.8% (17 of 445) of the patients. Influenza A/H1N1 pdm, A/H3N2, and influenza B co-circulated between week 36 (September 2014) and week 13 (March 2015); weeks 26 to 39 (June to September 2015) were dominated by A/H3N2 with sporadic detections of influenza B viruses (Figure 2).

RSV was exclusively detected in September 2014 and 2015 within the rainy season ($p < .001$). RSV strains were typed as RSV-A (17 of 31, 54.8%) and RSV-B (12 of 31, 38.7%) by PCR. All RSV-A strains belonged to the ON1 genotype (Figure S1). The genetic similarity between Lao strains ranged from 98.2% to 100% over the complete G gene sequence. All RSV-B sequences clustered within the BA9 genotype and were distinct from BA9 strains that were circulating in the country in 2010.[21] The THB/CB1 genotype previously identified in Lao PDR in 2010[21] was not observed in this study.

hMPV was detected almost all year round without any temporal variation ($p = .738$). hMPV strains were typed as genotype A2b (12 of 17, 70.6%), B1 (1 of 17, 5.9%) and B2 (4 of 17, 23.5%) based on partial F gene sequences (Figures 3A and S2A) and confirmed based on partial G gene sequences (Figure S2B). No temporal variation of strain circulation was observed and A2b, B1, and B2 strains were co-circulating: for instance, all three genotypes were detected in August 2015 (Figures 3A and S2).

PIV3 ($p < .001$) and hCoV-HKU1 ($p = .002$) were detected more frequently during the dry season. No seasonal differences were observed for the other viruses ($p = .103$ to 0.995).

4 | DISCUSSION

In Lao PDR, limited disease surveillance data prompted us to investigate and characterize a wide range of respiratory pathogens. Here we show that respiratory viral infections occur throughout the year in Lao PDR, with hRV being the most frequently detected virus (29.9% overall and 75.0% of co-infections; Figure 1). High frequencies of hRV infections were also reported in other studies carried out in Lao PDR, regardless of the population investigated.[3,11,12,22] Two studies found that hRV was the most frequently detected virus in all age groups in patients with ILI or acute lower respiratory infections.[11,12] hRV was also the second most frequent virus reported, after RSV, in children with severe respiratory acute infection.[22] Although other studies carried out elsewhere have shown that hRV can be found in 10%–33% of asymptomatic children[23–25] and has typically been associated with

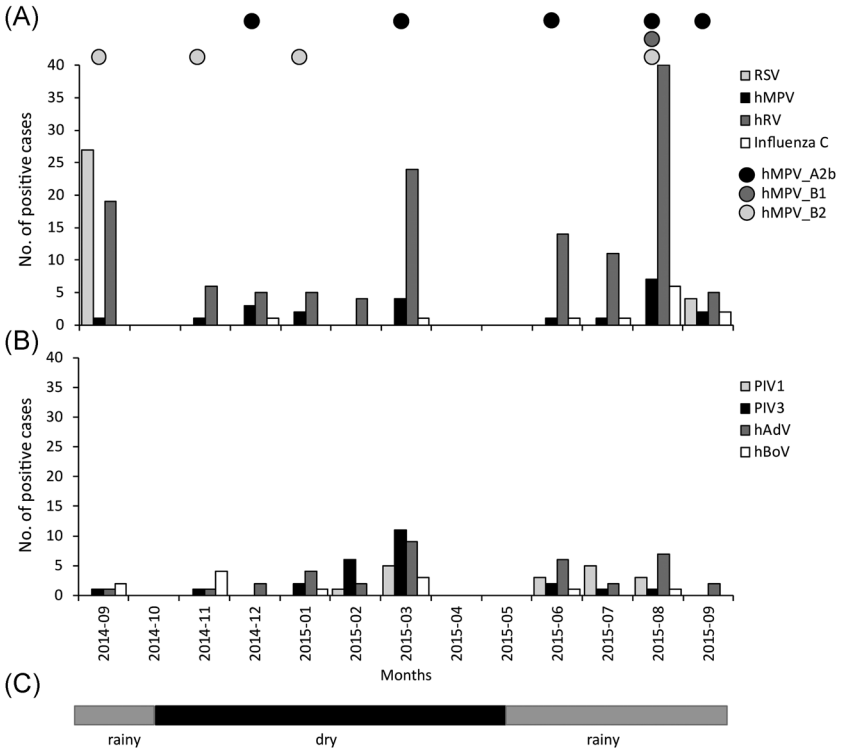

FIGURE 3 (A, B) Virus detection by months and (C) incidence in the rainy and dry season in 2014–2015 in Vientiane. No samples were collected in October 2014 and April–May 2015. hMPV genotypes detected each month are indicated on top of (A)

upper respiratory tract infections, its implication in more severe diseases such as bronchiolitis, pneumonia, or asthma exacerbation has also been suggested.[26] Co-infections tend to increase illness duration: hospital stay for children with hRV-RSV co-infections has been shown to be significantly longer than children infected with RSV or hRV alone.[27] In addition, hRV-C may be more virulent than hRV-A and –B.[28,29] Therefore, the importance of hRV should not be underestimated and further investigations and molecular characterization of hRV strains in mild and severe respiratory infections in Lao PDR are warranted.

Temporal trends of influenza A and B viruses observed during our study were concordant with a peak of A/H3N2 circulation in September 2015 reported during ILI community surveillance in nearly 1000 selected households in Vientiane.[3] It also aligns with the sentinel surveillance in Lao PDR (FluNet), which found A/H1N1 pdm, A/H3N2, and influenza B co-circulating during January–June 2015, followed by a dominance of A/H3N2 during the second half of the year. Thus, our data and other surveillance activities revealed two influenza seasons of equal intensities in 2015. This situation contrasts with the overall reported pattern of influenza circulation with low but steady levels of transmission from January to August followed by a period of increased transmission between September and December (Figure S3).[2,21] Current recommendations for seasonal influenza vaccination in April–May in Lao PDR[30,31] seem adequate to protect the population during the main influenza period. Risk groups eligible for influenza vaccination in the country include pregnant women, the elderly, healthcare personnel, and people with comorbidities, but currently not children. Influenza vaccination in children older than 6 months is recommended in certain countries.[32,33] This is not only beneficial to reduce disease burden, especially in younger children at higher risk of severe complications, but also contributes to herd immunity, and less transmission in the community.[32] In addition, the non-negligible proportion of cases occurring outside the main influenza period (45% on average, 2011–2019), coupled with unusual years such as 2015, requires further long-term monitoring in the country and brings into question the existence of a seasonal pattern of influenza transmission in Lao PDR.

Our study also confirms the seasonality of RSV circulation in Lao PDR, with increased incidence in the rainy season.[12,21,22] However, we did not detect RSV cases outside September 2014 and 2015. This contrasts with previous studies suggesting that RSV is present year-round with a surge of cases during the rainy season.[22] Younger children had an increased chance of being positive for RSV and had an increased risk of hospital admission, again highlighting the contribution of RSV to respiratory disease severity in young children. hMPV, discovered only two decades ago, shares clinical and epidemiological characteristics with RSV.[34] It has been shown that most children are infected with hMPV before 5 years of age. hMPV infections lead to a wide range of clinical symptoms including frequent bronchiolitis and pneumonia, with a peak of hospitalization for children aged 6–12 months.[35] However, no association with age or hospital admission was observed in our study. Five main genotypes have been described so far, often co-circulating. Indeed, our study provides the first molecular characterization of hMPV in Lao PDR and shows co-circulation of 3 genotypes. The predominant genotype has been shown to change over time and is influenced by genotype-specific levels of immunity in the community, virus evolution and escape[36,37] and differential replication advantage rather than a difference in virulence.[38]

An analysis of 30 years of influenza surveillance data in France highlighted the existence of a group of seasons showing similar transmission patterns, in addition to single unusual seasons of transmission. Temporal divergences were also associated with different spatial dissemination patterns between regions.[39] Therefore, in our setting, patient recruitment in a wider network of study sites over a larger timespan is warranted to overcome the spatiotemporal limitations of this study where children from only one hospital were recruited over the course of one year. Enhanced country-wide longitudinal surveillance will allow the identification of long-term seasonal trends of virus circulation in Lao PDR, both at the viral species and genotypes/subtypes/serotypes levels. A more intensive sampling comparing in- and outpatients will also be needed to further understand the burden of viruses such as hRV, RSV, or hMPV.

In our study, we combined results from both nasopharyngeal aspirates and throat swabs. This approach allowed for improved sensitivity for identifying the etiological agents of respiratory infections in a pediatric population. Others have also found that multiple sampling in an adult population with lower respiratory tract manifestations revealed an improved sensitivity for virus detection.[40] Nasopharyngeal aspirates provided a significantly increased sensitivity for detection only for RSV. This contrasts with a comparison of nasal, throat or nasopharyngeal swabs for the detection of RSV in hospitalized children in Lao PDR that showed no statistical difference between all three sampling materials.[41] However, viral loads in throat swabs in that study were lower than in nasal or nasopharyngeal swabs,[41] which can influence detection rates by conventional PCR. Nevertheless, this approach is cumbersome in low resource settings. As the comparison of detection rates between the two types of samples revealed no statistical difference for the vast majority of viruses studied, surveillance of viral upper respiratory tract infections using a sampling technique requiring less professional skills such as throat or nasal swab collection, compared to nasopharyngeal aspirates, can be envisaged to cover larger areas of Lao PDR.

Antibiotics were prescribed to 20.3% of outpatients in the Children Hospital in Vientiane Capital. This contrasts with overall high levels of 52% of antibiotic prescription in primary care settings in low and middle-income countries.[7] In rural areas of China, antibiotics were prescribed in 37%–55% of consultations for upper respiratory tract infections, while doctor training and healthcare level influenced prescription levels.[42,43] The apparent low antibiotic prescription rate in our study likely results from higher expertise and awareness in the capital. Indeed, Quet et al.[10] showed a difference in perception of antibiotic overuse by medical doctors in the Vientiane capital compared to Luang Prabang and Khammouane provinces (82.8% vs. 61.4% and 56.9%, respectively).

Easier access to information such as through the Internet or training likely contributes to improved knowledge.

With an infection characterization rate of more than 70%, our data provide a detailed overview of the dynamics of respiratory pathogens in the study population. This information will be useful to raise awareness among local medical practitioners and may provide guidance in planning vaccination campaigns in the country. Given the importance of influenza A/B infections, seasonal influenza vaccination for young children in Lao PDR could help decrease the annual burden. However, other childhood vaccinations may have a higher priority. We also show permanent co-circulation of several respiratory viruses, contributing to ILI cases all year round. This highlights the need to take them into account for differential diagnosis of respiratory infections outside the influenza season. Coronaviruses were among the least frequently detected viruses, but their importance has changed with the emergence and worldwide spread of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). The risk of future seasonal circulation of SARS-CoV-2 now requires the integration of this new pathogen in the respiratory disease differential diagnosis. The broad spectrum of disease severity induced by SARS-CoV-2 infections further justifies a more systematic laboratory diagnostic approach towards respiratory viruses and the improvement of already existing vaccinations against respiratory pathogens such as influenza to decrease the overall burden of respiratory (co-) infections.

DATA AVAILABILITY STATEMENT

Nucleotide sequences associated with this study are available on the European Nucleotide Archive under the study accession number PRJEB42813 and sequence accession numbers LR999699-LR999752. The data that support the findings of this study are available upon reasonable request to the corresponding author, after obtaining permission from the National Ethics Committee for Health Research (NECHR) in the Lao PDR. The data are not publicly available due to privacy or ethical restrictions.

ACKNOWLEDGMENTS

We would like to thank the participants and the staff at the Children's Hospital, Vientiane. We also thank the staff and administration at Institut Pasteur du Laos for their support. The study was funded by the Luxembourg Ministry of Foreign Affairs and by the Luxembourg Institute of Health, "PaReCIDS II" grant.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Study design: Claude P. Muller, Konstantin Evdokimov, Somxay Billamay, Antony P. Black. *Participant recruitment*: Sodaly Mongkhoune, Somxay Billamay. *Laboratory testing*: Konstantin Evdokimov, Keoudomphone Vilivong, Chantal J. Snoeck, Kinnaly Xaydalasouk, Aurélie Sausy. *Data interpretation*: Chantal J. Snoeck, Konstantin Evdokimov, Judith M. Hübschen, Antony P. Black, Claude P. Muller. *Manuscript writing*: Chantal J. Snoeck, Konstantin Evdokimov, Judith M. Hübschen, Antony P. Black, Claude P. Muller.

ORCID

Chantal J. Snoeck http://orcid.org/0000-0002-0000-1850
Maude Pauly http://orcid.org/0000-0001-6428-3140
Judith M. Hübschen https://orcid.org/0000-0002-5001-2128
Antony P. Black https://orcid.org/0000-0003-0579-0702

REFERENCES

1. Tregoning JS, Schwarze J. Respiratory viral infections in infants: causes, clinical symptoms, virology, and immunology. *Clin Microbiol Rev*. 2010;23(1):74-98.
2. Khamphaphongphane B, Ketmayoon P, Lewis HC, et al. Epidemiological and virological characteristics of seasonal and pandemic influenza in Lao PDR, 2008-2010. *Influenza Other Respir Viruses*. 2013;7(3):304-311.
3. Rudge JW, Inthalaphone N, Pavlicek R, et al. Epidemiology and aetiology of influenza-like illness among households in metropolitan Vientiane, Lao PDR: a prospective, community-based cohort study. *PLOS One*. 2019;14(4):e0214207.
4. Harris AM, Hicks LA, Qaseem A. High value care task force of the American College of Physicians for the Centers for Disease Control and Prevention. Appropriate antibiotic use for acute respiratory tract infection in adults: advice for high-value care from the American College of Physicians and the Centers for Disease Control and Prevention. *Ann Intern Med*. 2016;164(6):425-434.
5. Heikkinen T, Jarvinen A. The common cold. *Lancet*. 2003;361(9351):51-59.
6. Morris DE, Cleary DW, Clarke SC. Secondary bacterial infections associated with influenza pandemics. *Front Microbiol*. 2017;8:1041.
7. Sulis G, Adam P, Nafade V, et al. Antibiotic prescription practices in primary care in low- and middle-income countries: a systematic review and meta-analysis. *PLOS Med*. 2020;17(6):e1003139.
8. Dethlefsen L, McFall-Ngai M, Relman DA. An ecological and evolutionary perspective on human-microbe mutualism and disease. *Nature*. 2007;449(7164):811-818.
9. Abt MC, Osborne LC, Monticelli LA, et al. Commensal bacteria calibrate the activation threshold of innate antiviral immunity. *Immunity*. 2012;37(1):158-170.
10. Quet F, Vlieghe E, Leyer C, et al. Antibiotic prescription behaviours in Lao People's Democratic Republic: a knowledge, attitude and practice survey. *Bull World Health Organ*. 2015;93(4):219-227.
11. Vongphrachanh P, Simmerman JM, Phonekeo D, et al. An early report from newly established laboratory-based influenza surveillance in Lao PDR. *Influenza Other Respir Viruses*. 2010;4(2):47-52.
12. Sentilhes AC, Choumlivong K, Celhay O, et al. Respiratory virus infections in hospitalized children and adults in Lao PDR. *Influenza Other Respir Viruses*. 2013;7(6):1070-1078.
13. World Health Organization. WHO information for molecular diagnosis of influenza virus - update. 2015. 60 p. https://www.who.int/influenza/gisrs_laboratory/molecular_diagnosis_influenza_virus_humans_update_201403rev201505.pdf. Accessed September 10, 2017.
14. Buecher C, Mardy S, Wang W, et al. Use of a multiplex PCR/RT-PCR approach to assess the viral causes of influenza-like illnesses in Cambodia during three consecutive dry seasons. *J Med Virol*. 2010;82(10):1762-1772.
15. Auburn H, Zuckerman M, Broughton S, Greenough A, Smith M. Detection of nine respiratory RNA viruses using three multiplex RT-PCR assays incorporating a novel RNA internal control transcript. *J Virol Methods*. 2011;176(1-2):9-13.

16. Parveen S, Sullender WM, Fowler K, Lefkowitz EJ, Kapoor SK, Broor S. Genetic variability in the G protein gene of group A and B respiratory syncytial viruses from India. *J Clin Microbiol*. 2006;44(9):3055-3064.
17. Ludewick HP, Abed Y, van Niekerk N, Boivin G, Klugman KP, Madhi SA. Human metapneumovirus genetic variability, South Africa. *Emerg Infect Dis*. 2005;11(7):1074-1078.
18. Snoeck CJ, Owoade AA, Couacy-Hymann E, et al. High genetic diversity of Newcastle disease virus in poultry in West and Central Africa: cocirculation of genotype XIV and newly defined genotypes XVII and XVIII. *J Clin Microbiol*. 2013;51(7):2250-2260.
19. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol*. 2013;30(12):2725-2729.
20. Mekong River Commission, Annual Mekong Flood Report 2015. 2018. p. 106.
21. Snoeck CJ, Ponghsavath V, Luetteke N, et al. Etiology of viral respiratory infections in Northern Lao People's Democratic Republic. *J Med Virol*. 2018;90(10):1553-1558.
22. Nguyen VH, Dubot-Pérès A, Russell FM, et al. Acute respiratory infections in hospitalized children in Vientiane, Lao PDR—the importance of respiratory syncytial virus. *Sci Rep*. 2017;7(1):9318.
23. Fry AM, Lu X, Olsen SJ, et al. Human rhinovirus infections in rural Thailand: epidemiological evidence for rhinovirus as both pathogen and bystander. *PLOS One*. 2011;6(3):e17780.
24. Nokso-Koivisto J, Kinnari TJ, Lindahl P, Hovi T, Pitkaranta A. Human picornavirus and coronavirus RNA in nasopharynx of children without concurrent respiratory symptoms. *J Med Virol*. 2002;66(3):417-420.
25. Singleton RJ, Bulkow LR, Miernyk K, et al. Viral respiratory infections in hospitalized and community control children in Alaska. *J Med Virol*. 2010;82(7):1282-1290.
26. Jacobs SE, Lamson DM St, George K, Walsh TJ. Human rhinoviruses. *Clin Microbiol Rev*. 2013;26(1):135-162.
27. Piedra PA. Prospective multicenter study of viral etiology and hospital length of stay in children with severe bronchiolitis. *Arch Pediatr Adolesc Med*. 2012;166(8):700-706.
28. Linsuwanon P, Payungporn S, Samransamruajkit R, et al. High prevalence of human rhinovirus C infection in Thai children with acute lower respiratory tract disease. *J Infect*. 2009;59(2):115-121.
29. Smuts HE, Workman LJ, Zar HJ. Human rhinovirus infection in young African children with acute wheezing. *BMC Infect Dis*. 2011;11:65.
30. Hirve S. *Seasonal Influenza Vaccine Use in Low and Middle Income Countries in the Tropics and Subtropics. A systematic review*. Geneva, Switzerland: WHO; 2015:96.
31. Saha S, Chadha M, Al Mamun A, et al. Influenza seasonality and vaccination timing in tropical and subtropical areas of southern and south-eastern Asia. *Bull World Health Organ*. 2014;92(5):318-330.
32. European Centre for Disease Prevention and Control. Seasonal influenza vaccination and antiviral use in EU/EEA Member States – Overview of vaccine recommendations for 2017–2018 and vaccination coverage rates for 2015–2016 and 2016–2017 influenza seasons. 2018. https://www.ecdc.europa.eu/sites/default/files/documents/seasonal-influenza-antiviral-use-2018.pdf. Accessed July 5, 2020.
33. Grohskopf LA, Sokolow LZ, Broder KR, et al. Prevention and control of seasonal influenza with vaccines. *MMWR Recomm Rep*. 2016;65(5):1-54.
34. Kahn JS. Epidemiology of human metapneumovirus. *Clin Microbiol Rev*. 2006;19(3):546-557.
35. Shafagati N, Williams J. Human metapneumovirus—what we know now. *F1000Res*. 2018;7:135.
36. Matsuzaki Y, Itagaki T, Abiko C, Aoki Y, Suto A, Mizuta K. Clinical impact of human metapneumovirus genotypes and genotype-specific seroprevalence in Yamagata, Japan. *J Med Virol*. 2008;80(6):1084-1089.
37. Chung JY, Han TH, Kim SW, Hwang ES. Genotype variability of human metapneumovirus, South Korea. *J Med Virol*. 2008;80(5):902-905.
38. Zhou Z, Zhang P, Cui Y, et al. Experiments investigating the competitive growth advantage of two different genotypes of human metapneumovirus: implications for the alternation of genotype prevalence. *Sci Rep*. 2020;10(1):2852.
39. Coletti P, Poletto C, Turbelin C, Blanchon T, Colizza V. Shifting patterns of seasonal influenza epidemics. *Sci Rep*. 2018;8(1):12786.
40. Lieberman D, Lieberman D, Shimoni A, Keren-Naus A, Steinberg R, Shemer-Avni Y. Identification of respiratory viruses in adults: nasopharyngeal versus oropharyngeal sampling. *J Clin Microbiol*. 2009;47(11):3439-3443.
41. Nguyen VH, Russell FM, Dance DA, et al. Nasal or throat sampling is adequate for the detection of the human respiratory syncytial virus in children with acute respiratory infections. *J Med Virol*. 2019;91(9):1602-1607.
42. Sun Q, Dyar OJ, Zhao L, et al. Overuse of antibiotics for the common cold - attitudes and behaviors among doctors in rural areas of Shandong Province, China. *BMC Pharmacol Toxicol*. 2015;16:6.
43. Zhang Z, Hu Y, Zou G, et al. Antibiotic prescribing for upper respiratory infections among children in rural China: a cross-sectional study of outpatient prescriptions. *Glob Health Action*. 2017;10(1):1287334.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Snoeck CJ, Evdokimov K, Xaydalasouk K, et al. Epidemiology of acute respiratory viral infections in children in Vientiane, Lao People's Democratic Republic. *J Med Virol*. 2021;93:4748-4755. https://doi.org/10.1002/jmv.27004