Respiratory Viral Infections and Their Association with Acute Febrile Illness Within Nicaragua

by

Damion Paul Brown

Duke Global Health Institute
Duke University

Date:_____
Approved:

_____
Megan Reller, Supervisor

_____
Joe Egger

_____
Lindsay Saunders

Thesis submitted in partial fulfillment of
the requirements for the degree of
Master of Science in the Duke Global Health Institute
in the Graduate School of Duke University

2023

ABSTRACT

Respiratory Viral Infections and Their Association with Acute Febrile Illness Within Nicaragua

by

Damion Paul Brown

Duke Global Health Institute
Duke University

Date:_____
Approved:

_____
Megan Reller, Supervisor

_____
Joe Egger

_____
Lindsay Saunders

An abstract of a thesis submitted in partial
fulfillment of the requirements for the degree
of Master of Science in the Duke Global Health Institute
in the Graduate School of Duke University

2023

Copyright by
Damion Paul Brown
2023

Abstract

Acute febrile illness (AFI) is defined as a non-localized fever of ≥ 38°C and affects many individuals around the world. Due to many different diseases causing AFI, it can often be difficult to diagnose patients with the correct etiology. As such, it is hypothesized that a significant percentage of patients within Leon, Nicaragua are often misdiagnosed with ailments such as dengue fever when a respiratory virus is the etiologic agent causing the patient's AFI. Two-hundred sixty-two nasopharyngeal swabs from patients ≧ 1 year old were obtained between January 2021 and March 2022 at the Hospital Escuela Oscar Danilo Rosales Arguello (HEODRA) located in Leon, Nicaragua. The DNA and/or RNA of each sample was extracted using the Qiagen EZ1 Advanced XL alongside the EZ1 virus kit. After DNA/RNA extraction the samples were then sequenced via real-time polymerase chain reaction (RT-PCR) on the Luminex Integrated System NxTAG Respiratory Pathogen Panel (RPP) platform which detects a multitude of respiratory pathogens. The RPP panel results were then cross-referenced with each patient's sociodemographics, clinical symptoms, and end diagnosis. Around 20% of patient samples tested positive on the RPP in which rhinovirus/enterovirus and influenza A were the most detected targets. These patients were often younger and more likely to be male, work in an office, have a shorter fever and rhinitis, and be diagnosed

with dengue. These results support the hypothesis as well as show differences in symptoms that providers can use to better diagnose patients.

Dedication

This thesis is dedicated to my sister who passed away last year as well as everyone else who believed in me throughout the entire process. Rest in peace Kaylee and thank you everyone.

Contents

List of Tables

List of Figures

Acknowledgements

I gratefully acknowledge the productive partnership between Nicaraguan PIs Dr. Filemon Bucardo and Dr. Armando Matute and Duke PI Dr. Megan Reller, my mentor.

I also want to acknowledge Dr. Brad Nicholson who graciously allowed me to utilize his lab space and equipment at the Durham, North Carolina VA. I would like to thank Dr. Megan Reller, Dr. Joe Egger, Dr. Lindsay Saunders for being a part of my thesis committee. Additionally, I would like to thank Coralei Neighbors, Tianchen Sheng, and Alexis Domeracki for helping with data analysis.

Finally, I would like to recognize the participants, without whose help the research could not have been completed.

1. Introduction

Acute febrile illness (AFI) is a common cause of illness and hospitalization worldwide, especially in the tropics [1]. AFI is defined as non-localized (undifferentiated) fever, or fever without focus. The etiology of AFI varies greatly amongst geographic regions, climates, and seasons [2, 5]. Since the clinical presentation of AFI is non-specific, on-site confirmatory diagnostic testing is needed to guide management of patients, including proper treatment.

Sentinel studies by Dr. Reller and colleagues in Sri Lanka in 2007 found unsuspected leptospirosis and rickettsioses were more common (14% and 18%, respectively) than dengue (5%), although dengue was more often suspected. Since respiratory symptoms were especially common in those without a diagnosis of AFI after comprehensive testing, her group evaluated the proportion of AFI attributable to respiratory viruses in a follow-up study of AFI in 2012-4. That study found 21.1% of participants were positive for a respiratory virus among admitted participants ≥ 1 year of age presenting with AFI. Of these patients, more than half were prescribed antibiotics [2-4].

Similarly in Latin America, including Nicaragua, much research has focused on arboviral etiologies of AFI, including Dengue virus (DENV), Chikungunya Virus (CHIKV), and more recently Zika Virus (ZIKV) [5-9]. In a comprehensive study of AFI in

2008 in Nicaragua, Dr. Reller's group identified unrecognized bacterial etiologies of AFI, including leptospirosis, Q fever, *E. chaffeensis*, and Rickettsia infections in addition to dengue [10-13]. Since respiratory symptoms were common among the undiagnosed, nasopharyngeal swabs were collected in the 2018 follow-up study to test the hypothesis that a significant percentage of undiagnosed AFI in Nicaragua would also be attributed to respiratory viruses. We describe here the importance of respiratory viral infections as causes of AFI in Nicaragua.

2. Methods

2.1 Setting

Participants were recruited at the Hospital Escuela Oscar Danilo Rosales Arguello (HEODRA), a teaching hospital within the Universidad Nacional Autonoma de Nicaragua (UNAN) within Leon, Nicaragua. HEODRA's capacity is approximately 400 inpatient beds, with both pediatric and adult services. Participants also were recruited from 1 of 2 outpatient clinics within the area: Perla Maria or Felix P. Picado.

2.2 Participants

Eligible participants were ≥ 1 year old who presented with AFI to the prior locations between January 2021 and March 2022. A written informed consent was obtained from each patient or from the parents or guardians of participants under 18 years of age prior to sample collection.

2.3 Respiratory Pathogen Testing

Flocked nasopharyngeal (NP) swab samples were collected and stored in viral transport media and subsequently frozen at -80oC. Samples were shipped on dry ice to Durham, North Carolina from Nicaragua. Each sample was prepared for nucleic acid extraction using the EZ1 virus kit. The nucleic acids from each of the samples were then extracted using the Qiagen EZ1 Advanced XL. Extracted samples (200 mL) were eluted into the buffer and stored at -80oC. The viral DNA/RNA was then amplified utilizing

integrated polymerase chain reaction and bead hybridization with a protocol outlined in the Luminex Integrated System NxTAG Respiratory Pathogen Panel (RPP) platform. After amplification, the samples were then loaded onto the RPP to determine the presence of respiratory virus DNA/RNA. The RPP detects a wide range of viral and bacterial targets that can cause AFI: Influenza A, Influenza A H1/H3, Influenza B, Rhinovirus/Enterovirus, Adenovirus, Parainfluenza virus 1-4, Coronavirus HKU1/NL63/229E/OC43, Respiratory Syncytial Virus A/B, Human Metapneumovirus (hMPV), Human Bocavirus (HBoV), *Chlamydophila pneumoniae, Mycoplasma pneumonia*e, and *Legionella pneumophila*.

2.4 Statistical Analysis

Sociodemographic and clinical data from RPP positive and RPP negative participants were analyzed with Fisher's exact test for categorical variables and Kruskal-Wallis test for continuous variables. In order to determine the odds ratio (OR) and confidence intervals (CI) of continuous variables, logistic regression was performed. Tachypnea was categorized as >40 breaths per minute for children aged 1-5 and >20 breaths per minute for participants aged ≥ 5 years old [14]. Tachycardia was categorized as 180 beats per minute for children < 2 years age, 140 beats per minute for children aged between 2-<6, 130 beats per minute for children aged 6-<13, 110 beats per minute for children aged 13-<18, and 100 beats per minute for participants aged >=18 years of age

[15]. The program R, version 4.2.1 (R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/) was used for all statistical analyses.

2.5 Ethical Statement

Study procedures were explained to participants and participants were given time to ask questions. Consent signatures or thumbprints were obtained from participants 18 years or older or legal guardians of participants ages 1-17 years in addition to assent from participants 12-17 years. This study was approved by the Duke University Health's Institutional Review Board (Pro00014461) as well as the UNAN-Leon Ethics Committee for Biomedical Studies (FWA00004523/IRB00003342).

3. Results

3.1 Cohort and RPP testing results

Nasopharyngeal specimens were obtained from a total of 262 participants and tested on the RPP platform. The participants had an overall median age of 29.0 IQR (17.0 - 44.0) years and 15 (6%) of those participants were <5 years of age. Slightly more than half (134 (51%)) of the participants were male. Of these, 1 patient received an invalid result on the RPP platform, thus was removed from future analysis.

A total of 51 (19.5%) participants tested positive for one or more of the viruses on the RPP panel (Table 1). The predominant respiratory virus detected in adults and children was Rhinovirus/Enterovirus (21, 8% of all participants tested). Influenza A was the second most frequently detected virus (9, 3% of all participants tested). Other viruses, such as Bocavirus (2%), Parainfluenza virus (2%), Adenovirus (2%), Coronavirus (1%), and Human Metapneumovirus (0.4%) were also detected.

A minority of participants (3, 1%) were positive for more than one viral target. Those participants tested positive for: Influenza A H3/Adenovirus, Rhinovirus/Enterovirus/Adenovirus, and Rhinovirus/Enterovirus/Bocavirus respectively. Participants <5 years of age were more likely (p=0.01) to have a positive RPP result (Figure 1).

Table 1. Respiratory Viruses Detected Among 262 participants with Acute Febrile Illness in Nicaragua, 2021-2022.

Virus Type	All ages N=262 (%)	Patients ≧5 years N=245	Patients <5 years N=15	*P* value
Rhinovirus/Enterovirus	21 (8)	19 (8)	2 (13)	0.35
Influenza A H3	8 (3)	7 (3)	1 (7)	0.38
Bocavirus	5 (2)	4 (2)	1 (7)	0.26
Adenovirus	4 (2)	2 (0.8)	2 (13)	0.02
Coronavirus NL63	2 (0.8)	2 (0.8)	-	
Parainfluenza 3	2 (0.8)	2 (0.8)	-	
Parainfluenza 1	1 (0.4)	1 (0.4)	-	
Parainfluenza 4	1 (0.4)	1 (0.4)	-	
Parainfluenza 5	1 (0.4)	1 (0.4)	-	
Metapneumovirus	1 (0.4)	1 (0.4)	-	
Coronavirus HKU1	1 (0.4)	1 (0.4)	-	
Influenza A (unknown subtype)	1 (0.4)	1 (0.4)	-	
More than 1 virus detected	3 (1)	2 (0.8)	1 (7)	0.16
Positive for >1 virus	51(19.5)	44 (18)	7 (46.7)	0.01

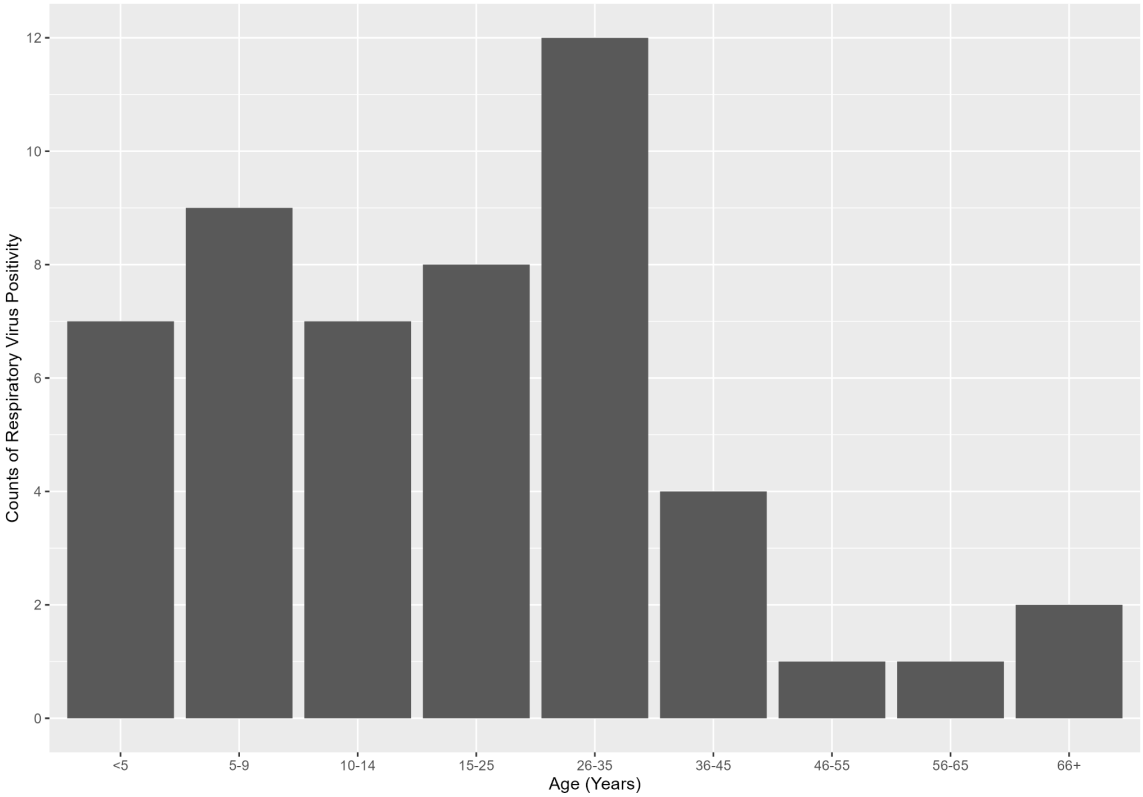

Figure 1. Number of respiratory viruses identified by age group, Leon, Nicaragua between January 2021 and April 2022.

3.2 Seasonality and Monthly Data

The absolute number and proportion of nasopharyngeal samples tested that were positive varied by month. (Figure 2). The proportion positive was greatest in February 2021 - March 2021. The months in which the greatest number of nasopharyngeal specimens were obtained and tested were August 2021 and September 2021.

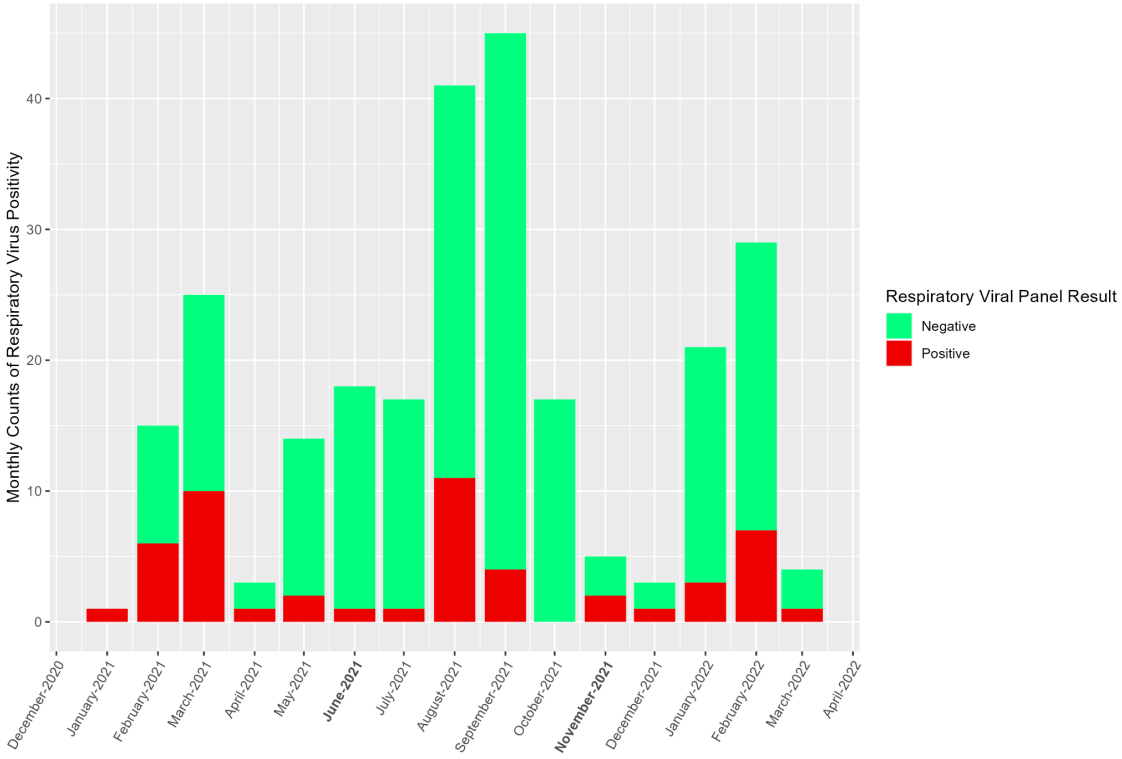

Figure 2. Results of respiratory viral testing by month, January 2021 -April 2022, Leon, Nicaragua.

3.3 Sociodemographic and Epidemiological data

Sociodemographic and epidemiologic data for participants tested for respiratory viruses can be found in Table 2. Participants who tested positive for respiratory viruses were significantly younger (median age of 21.0 vs median age of 31.5 years, $p < 0.01$). Positive participants were also more likely to be male (OR 2.4, 95% CI: 1.21, 4.95) and were more likely to work in an office (OR 2.53, 95% CI: 0.97, 6.45).

There were no other significant differences between participants who tested positive versus negative for respiratory viruses with respect to reported occupation, education level, residence, sick contacts, recent travel, smoking, or prior antibiotic usage

Table 2. Epidemiologic and Sociodemographic Characteristics of Participants with Acute Febrile Illness Tested for Respiratory Viruses, Leon Nicaragua, January 2021 - April 2022.
*** Odds Ratio/CI obtained with logistic regression.**

	Number (%) or Median (IQR) Respiratory Pathogen Panel Negative	Number (%) or Median (IQR) Respiratory Pathogen Panel Positive	*P* value	Odds Ratio (95% CI)
Age	31.5 (20-46.2)	21.0 (7.5-31.5)	<0.01	0.96 (0.94, 0.98)*
Male	99 (47)	35 (69)	0.01	2.4 (1.21, 4.95)
Occupation:				
House	58 (35)	6 (22)	0.27	0.53 (0.17, 1.45)
Industrial worker	6 (4)	2 (7)	0.31	2.11 (0.20, 12.68)
Agricultural				
Student	4 (2)	1 (4)	0.54	1.54 (0.03, 16.42)
Business	25 (15)	3 (11)	0.77	0.70 (0.13, 2.58)
Office	15 (9)	-		-
Other	35 (21)	11 (41)	0.05	2.53 (0.97, 6.45)
	22 (13)	4 (15)	0.77	1.13 (0.26, 3.77)
>=12th grade education (adults)	98 (59)	18 (67)	0.67	1.26 (0.50, 3.41)
Residence:				
Urban	182 (87)	47 (92)	0.47	1.68 (0.54, 6.93)
Rural	26 (12)	4 (8)		

	Number (%) or Median (IQR) Respiratory Pathogen Panel Negative	Number (%) or Median (IQR) Respiratory Pathogen Panel Positive	*P* value	Odds Ratio (95% CI)
Sick Contact within 4 weeks	72 (34)	20 (10)	0.52	0.81 (0.41, 1.61)
Travel in the past 4 weeks	58 (25)	13 (28)	0.86	1.11 (0.53, 2.45)
Smoking: - Smoker - Previously Smoked - Never Smoked	 19 (9) 22 (10) 163 (78)	 6 (12) 4 (8) 39 (76)	 0.59 0.79	 1.36 (0.42, 3.81) 0.74 (0.18, 2.32)
Prior Antibiotic use	7 (3)	5 (10)	0.64	2.06 (0.22, 29.41)

3.4 Clinical Characteristics

Clinical characteristics of participants who tested positive or negative for respiratory viruses are shown in Table 3. The only statistically significant differences within the clinical features between participants who were RPP positive vs RPP negative were oxygen saturation (99% IQR 97.25-99 vs IQR 96.75-98) and conjunctivitis (OR 12.64, 95% CI: 0.99, 672.89).

Table 3. Clinical Features of Participants with Acute Febrile Illness Tested for Respiratory Viruses Nicaragua, January 2021 - April 2022.
*** Odds Ratio/CI obtained with logistic regression.**

Clinical Features:	Number (%) or Median (IQR) Respiratory Pathogen Panel Negative	Number (%) or Median (IQR) Respiratory Pathogen Panel Positive	*P* value	Odds Ratio (95% CI)
Days of fever	3 (2-4)	2 (1-4)	0.07	0.81 (0.66, 0.96)*
Rhinitis	101 (48)	32 (63)	0.09	1.78 (0.91, 3.55)
Cough	126 (60)	34 (67)	0.52	1.30 (0.66, 2.65)
Sore Throat	96 (46)	28 (55)	0.35	1.41 (0.73, 2.74)
Dyspnea	41 (20)	7 (14)	0.42	0.66 (0.23, 1.63)
Chills	131 (62)	34 (67)	0.75	1.16 (0.58, 2.37)
Weight Loss	16 (8)	3 (6)	1	0.75 (0.13, 2.77)
Bloody Sputum	-	1 (2)	0.20	-
Vomiting	52 (25)	9 (18)	0.36	0.66 (0.26, 1.49)
Diarrhea	60 (29)	11 (22)	0.38	0.68 (0.29, 1.46)
Abdomen pain	74 (35)	18 (35)	1	0.99 (0.49, 1.95)
Dysuria	28 (13)	4 (8)	0.35	0.55 (0.13, 1.68)
Decrease in diuresis	20 (10)	5 (10)	1	1.02 (0.28, 3.01)
Headache	161 (77)	35 (69)	0.20	0.64 (0.31, 1.35)

Fatigue	47 (22)	7 (14)	0.18	0.55 (0.19, 1.33)
Seizure	3 (1)	-	-	-
Joint Pain	114 (54)	25 (49)	0.53	0.79 (0.41, 1.45)
Muscle Ache	112 (53)	26 (51)	0.76	0.89 (0.46, 1.73)
Rash	6 (3)	2 (4)	0.65	1.40 (0.13, 8.15)
Allergies	4 (2)	2 (4)	0.34	2.05 (0.18, 14.81)
Conjunctivitis	1 (.5)	3 (6)	0.03	12.64 (0.99, 672.89)
Retroorbital pain	94 (45)	16 (316)	0.08	0.56 (0.27, 1.11)
Ear pain	17 (8)	3 (6)	0.77	0.70 (0.13, 2.58)
Hearing Loss	5 (2)	3 (6)	0.19	2.53 (0.38, 13.5)
Epistaxis	-	1 (2)	0.20	-
Loss of Taste	38 (18)	5 (10)	0.21	0.50 (0.14, 1.37)
Loss of Smell	43 (20)	6 (12)	0.17	0.51 (0.17, 1.32)
Admission Temp (C)	36.75 (36.30-37.60)	36.70 (36.35-37.9)	0.97	1.01 (0.71, 1.40)*
Tachycardia on Exam	42 (20)	7 (14)	0.42	0.64 (0.23, 1.57)
Tachypnea	65 (31)	16 (31)	1	1.01 (0.49, 2.05)
Oxygen Saturation	98 (96.75-98)	98 (97.25-99)	0.03	1.14 (0.97, 1.37)*
Conjunctival Injection	1 (.5)	2 (4)	0.10	8.43 (0.43, 503.83)
Throat	29 (14)	12 (24)	0.13	1.92 (0.82, 4.29)

Inflammation				
Enlarged Lymph Nodes	198 (94)	47 (92)	0.53	0.71 (0.20, 3.17)
Crepitations	20 (10)	5 (10)	1	1.03 (0.29, 3.04)
Rhonchi	13 (6)	3 (6)	1	0.94 (0.17, 3.62)
Wheezing	5 (2)	2 (4)	0.63	1.67 (0.15, 10.57)

3.5 Clinical Management of Patients

Clinical diagnoses of participants were evaluated to compare clinical diagnoses (without diagnostic testing) with RPP results. Participants who were RPP positive were more likely to receive a clinical diagnosis of dengue compared with participants who were RPP negative (OR 2.64, 95% CI: 1.29, 5.36). Participants who were RPP positive were less likely to be clinically diagnosed with COVID-19 compared with participants who were RPP negative (OR 0.39, 95% CI: 0.20, 0.77). Additionally, participants who were RPP positive had a greater median white blood cell (WBC) count (median 8095, IQR: 6000-10112.5) than participants who were RPP negative (median 6325, IQR: 4900-9282.5).

Table 4. Clinical Diagnoses of Participants with Acute Febrile Illness Tested for Respiratory Viruses Nicaragua, January 2021 - April 2022.
*** Odds Ratio/CI obtained with logistic regression.**

Clinical Diagnosis at Presentation with AFI	Number (%) or Median (IQR) Respiratory Pathogen Panel Negative	Number (%) or Median (IQR) Respiratory Pathogen Panel Positive	*P* value	Odds Ratio (95% CI)
Viral				
- Dengue	41 (20)	20 (39)	0.01	2.64 (1.29, 5.36)
- Flu	2 (1)	2 (4)	0.17	4.21 (0.30, 59.47)
- Febrile Syndrome	58 (28)	18 (35)	0.30	1.43 (0.70, 2.85)
- COVID	142 (68)	23 (45)	<0.01	0.39 (0.20, 0.77)
- Other	2 (1)	1 (2)	0.48	2.07 (0.03, 40.56)
Bacteria				
- Bacteremia	1 (.5)	-	-	-
- Leptospirosis	-	-	-	-
- Tuberculosis	-	-	-	-
- Localized	43 (20)	10 (20)	1	0.95 (0.39, 2.12)
- Other	8 (4)	1 (2)	0.69	0.45 (0.01, 3.36)
Parasite				
- Malaria	4 (2)	-	-	-
- Other	2 (1)	-	-	-
Antibiotic Administered:	56 (27)	16 (31)	0.49	1.26 (0.60, 2.55)
- Beta Lactam	15 (7)	6 (12)	0.26	1.73 (0.52, 5.05)
- 3rd gen cephalosporin	7 (3)	1 (2)	1	0.58 (0.01, 4.69)
- 4th gen cephalosporin	1 (0.5)	-	-	-
- Fluoroquinolones	2 (1)	-	-	-
- Macrolides	33 (16)	9 (18)	0.83	1.15 (0.45, 2.70)
- Anti-viral	1 (0.5)	1 (2)	0.35	4.15 (0.05, 328.73)

Clinical Diagnosis at Presentation with AFI	Number (%) or Median (IQR) Respiratory Pathogen Panel Negative	Number (%) or Median (IQR) Respiratory Pathogen Panel Positive	*P* value	Odds Ratio (95% CI)
Abnormal X-ray	11 (5)	1 (2)	0.47	0.36 (0.01, 2.61)
WBC count	6325 (4900-9282.5)	8095 (6000-10112.5)	0.04	1.00 (1.00, 1.00)*
CRP performed	32 (15)	10 (20)	0.44	3.10 (0.04, 258.26)

4. Discussion

In this cohort with acute febrile illness in Nicaragua, nearly 20% of the patient samples tested on the RPP platform tested positive for a respiratory virus. Participants within our study population who tested positive for respiratory virus were typically younger, male, and, if adults, more often employed in an office. Adenovirus and more than 1 respiratory virus were more common in those <5 years. The months of June through to November are considered peak months for influenza-like illness for Nicaragua [16]. The majority of the NP swabs that were tested on the RPP were collected between this period (Figure 2). Interestingly, the proportion of positive RPP tests to negative RPP tests remains high outside of this period.

The majority of studies done on respiratory viruses in Nicaragua are limited to Influenza A/B and RSV. While Influenza A was one of the most predominant viral targets detected in the study, it may be of interest to public health researchers and epidemiologists to broaden the focus of respiratory viruses in Nicaragua to include Rhinovirus/Enterovirus, Bocavirus, and Adenovirus as these were the most commonly detected targets within our study.

Due to the similar clinical presentations of respiratory viral illnesses and other causes of AFI (Table 3), diagnostic testing is important. The initial hypothesis that participants with AFI are often misdiagnosed with ailments such as dengue is supported

by the data from this study. Nearly 39% of respiratory virus-positive participants were thought to have dengue fever.

Additionally, those with respiratory viruses were as likely to receive an antibiotic as other patients. On-site diagnosis of respiratory virus infection could decrease unnecessary antibiotic use.

4.1 Study strengths and limitations

Proving causality that the viral targets detected were the root cause of the patient's AFI is not something that could be evaluated in this study. First, comprehensive testing for other AFI agents has not yet been completed for these patients. Second, some of the respiratory viruses identified can be found in asymptomatic patients. Therefore, it is possible that some of the participants who were RPP positive were colonized with the respiratory virus and the virus was not the cause of the AFI. One way to assess the clinical importance of respiratory viruses identified would be a case-control study in which age-matched controls without infectious symptoms were also tested to see if there were differences between the likelihood of identifying respiratory viruses between the two groups.

Another limitation is that respiratory viral samples could only be obtained in a subset of patients with AFI. It is harder to get respiratory viral samples from young

children, since parents can be reluctant to consent to this safe, but uncomfortable, procedure. We found that those individuals who had respiratory virus samples collected were statistically significantly different from those without respiratory samples with respect to age and sex (Table 2).

Additionally, this study took place during the SARS-CoV-2 pandemic. The RPP platform that samples were tested on did not detect this viral target. Several participants who tested negative on the RPP may have been experiencing an infection from SARS-CoV-2. A recent study showed that, among 8 urban health centers in Nicaragua, the prevalence of SARS-CoV-2 was approximately 14% during the time period of our study [17].

It is also important to note the relatively low sample size of this study. With a greater sample size, we would have had greater power to detect associations between clinical features and respiratory virus positivity (Table 3). It is also possible that the differences we observed may have been due to chance alone.

4.2 Implications for policy and practice

Assessment of the epidemiology of respiratory viruses in Nicaragua could inform interventions, such as influenza vaccination and treatment of high-risk groups.

Additionally, respiratory viral testing at point of care could limit unnecessary antibiotic use.

4.3 Implications for further research

It is clear that additional focus needs to be brought upon respiratory viruses and their relative importance in patients with AFI. By furthering the understanding of AFI as a whole, providers will be able to more accurately manage participants who present with AFI. It may be of interest to evaluate protocol within emergency departments or outpatient clinics in which a respiratory viral test is ordered when patients present. One could also study whether point of care diagnosis of influenza limits antimicrobial prescribing.

5. Conclusion

This study found that respiratory viruses were present in 19% of patients presenting with AFI in Leon, Nicaragua who were tested for respiratory viruses. Additional research needs to be done to assess the clinical importance of these infections, as well as the implications for preventive strategies and appropriate antibiotic use. Ultimately the goal is to improve patient outcomes via enhanced clinical decision making and to improve antimicrobial stewardship.

6. References

1. Rhee, C., Kharod, G. A., Schaad, N., Furukawa, N. W., Vora, N. M., Blaney, D. D., Crump, J. A., & Clarke, K. R. (2019). Global knowledge gaps in acute febrile illness etiologic investigations: A scoping review. PLoS neglected tropical diseases, 13(11), e0007792. https://doi.org/10.1371/journal.pntd.0007792

2. Tillekeratne, L Gayani et al. "Respiratory Viral Infection: An Underappreciated Cause of Acute Febrile Illness Admissions in Southern Sri Lanka." The American journal of tropical medicine and hygiene vol. 100,3 (2019): 672-680. doi:10.4269/ajtmh.18-0699

3. Reller, M. E., Bodinayake, C., Nagahawatte, A., Devasiri, V., Kodikara-Arachichi, W., Strouse, J. J., Flom, J. E., Østbye, T., Woods, C. W., & Dumler, J. S. (2012). Unsuspected rickettsioses among patients with acute febrile illness, Sri Lanka, 2007. Emerging infectious diseases, 18(5), 825–829. https://doi.org/10.3201/eid1805.111563

4. Reller, M. E., Bodinayake, C., Nagahawatte, A., Devasiri, V., Kodikara-Arachichi, W., Strouse, J. J., Flom, J. E., Dumler, J. S., & Woods, C. W. (2011). Leptospirosis as frequent cause of acute febrile illness in southern Sri Lanka. Emerging infectious diseases, 17(9), 1678–1684. https://doi.org/10.3201/eid1709.100915

5. Moreira, J et al. "Epidemiology of acute febrile illness in Latin America." Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases vol. 24,8 (2018): 827-835. doi:10.1016/j.cmi.2018.05.001

6. Burger-Calderon, R., Bustos Carrillo, F., Gresh, L., Ojeda, S., Sanchez, N., Plazaola, M., Katzelnick, L., Mercado, B. L., Monterrey, J. C., Elizondo, D., Arguello, S., Nuñez, A., Gordon, A., Balmaseda, A., Kuan, G., & Harris, E. (2020). Age-dependent manifestations and case definitions of paediatric Zika: A prospective cohort study. The Lancet. Infectious Diseases, 20(3), 371–380. https://doi.org/10.1016/S1473-3099(19)30547-X

7. Reller, M. E., de Silva, A. M., Miles, J. J., Jadi, R. S., Broadwater, A., Walker, K., Woods, C., Mayorga, O., & Matute, A. (2016). Unsuspected Dengue as a Cause of Acute Febrile Illness in Children and Adults in Western Nicaragua. PLoS Neglected Tropical Diseases, 10(10), e0005026.

https://doi.org/10.1371/journal.pntd.0005026

8. Waggoner, J. J., Balmaseda, A., Gresh, L., Sahoo, M. K., Montoya, M., Wang, C., Abeynayake, J., Kuan, G., Pinsky, B. A., & Harris, E. (2016). Homotypic Dengue Virus Reinfections in Nicaraguan Children. The Journal of Infectious Diseases, 214(7), 986–993. https://doi.org/10.1093/infdis/jiw099

9. Waggoner, J. J., Gresh, L., Vargas, M. J., Ballesteros, G., Tellez, Y., Soda, K. J., Sahoo, M. K., Nuñez, A., Balmaseda, A., Harris, E., & Pinsky, B. A. (2016). Viremia and Clinical Presentation in Nicaraguan Patients Infected With Zika Virus, Chikungunya Virus, and Dengue Virus. Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America, 63(12), 1584–1590. https://doi.org/10.1093/cid/ciw589

10. Reller, M. E., Wunder, E. A. J., Miles, J. J., Flom, J. E., Mayorga, O., Woods, C. W., Ko, A. I., Dumler, J. S., & Matute, A. J. (2014). Unsuspected leptospirosis is a cause of acute febrile illness in Nicaragua. PLoS Neglected Tropical Diseases, 8(7), e2941. https://doi.org/10.1371/journal.pntd.0002941

11. Trevejo, R. T., Rigau-Pérez, J. G., Ashford, D. A., McClure, E. M., Jarquín-González, C., Amador, J. J., de los Reyes, J. O., Gonzalez, A., Zaki, S. R., Shieh, W. J., McLean, R. G., Nasci, R. S., Weyant, R. S., Bolin, C. A., Bragg, S. L., Perkins, B. A., & Spiegel, R. A. (1998). Epidemic leptospirosis associated with pulmonary hemorrhage-Nicaragua, 1995. The Journal of Infectious Diseases, 178(5), 1457–1463. https://doi.org/10.1086/314424

12. Reller, M. E., Chikeka, I., Miles, J. J., Dumler, J. S., Woods, C. W., Mayorga, O., & Matute, A. J. (2016). First Identification and Description of Rickettsioses and Q Fever as Causes of Acute Febrile Illness in Nicaragua. PLoS Neglected Tropical Diseases, 10(12), e0005185. https://doi.org/10.1371/journal.pntd.0005185

13. Chikeka, I., Matute, A. J., Dumler, J. S., Woods, C. W., Mayorga, O., & Reller, M. E. (2016). Use of Peptide-Based Enzyme-Linked Immunosorbent Assay followed by Immunofluorescence Assay To Document Ehrlichia chaffeensis as a Cause of Febrile Illness in Nicaragua. Journal of Clinical Microbiology, 54(6), 1581–1585. https://doi.org/10.1128/JCM.03331-15

14. Jain, S., Williams, D. J., Arnold, S. R., Ampofo, K., Bramley, A. M., Reed, C., Stockmann, C., Anderson, E. J., Grijalva, C. G., Self, W. H., Zhu, Y., Patel, A., Hymas, W., Chappell, J. D., Kaufman, R. A., Kan, J. H., Dansie, D., Lenny, N.,

Hillyard, D. R., Haynes, L. M., … CDC EPIC Study Team (2015). Community-acquired pneumonia requiring hospitalization among U.S. children. The New England journal of medicine, 372(9), 835–845. https://doi.org/10.1056/NEJMoa1405870

15. Goldstein, B., Giroir, B., Randolph, A., & International Consensus Conference on Pediatric Sepsis (2005). International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics. Pediatric critical care medicine : a journal of the Society of Critical Care Medicine and the World Federation of Pediatric Intensive and Critical Care Societies, 6(1), 2–8. https://doi.org/10.1097/01.PCC.0000149131.72248.E6

16. Hirve, S., Newman, L. P., Paget, J., Azziz-Baumgartner, E., Fitzner, J., Bhat, N., Vandemaele, K., & Zhang, W. (2016). Influenza Seasonality in the Tropics and Subtropics - When to Vaccinate?. PloS one, 11(4), e0153003. https://doi.org/10.1371/journal.pone.0153003

17. Huete-Pérez, J. A., Colgrove, R. C., Cabezas-Robelo, C., Páiz-Medina, L., Hunsajarupan, B., Silva, S., Quant, C., & Huete, A. (2022). SARS-CoV-2 prevalence at eight urban health clinics in Nicaragua: possible implications for the COVID-19 pandemic. IJID regions, 2, 110–117. https://doi.org/10.1016/j.ijregi.2021.12.013