Short Communication / Comunicación Corta

Molecular identification of respiratory viruses in Itapúa, Paraguay during the year 2023

*Karina Cáceres Fernández[1] iD **, Ramona Morales Rivé[1]** iD **, Sandra Caballero Gómez[2]** iD

[1]Universidad Autónoma de Encarnación. Facultad de Ciencias Químicas y Farmacéuticas. Paraguay
[2]Universidad Autónoma Encarnación, Hospital Regional de Encarnación. Paraguay

---

Cómo referenciar este artículo/ How to reference this article:	*Cáceres Fernández K, Morales Rivé R, Caballero Gómez S.* **Molecular identification of respiratory viruses in Itapúa, Paraguay during the year 2023**. *Mem. Inst. Investig. Cienc. Salud. 2024; 22(1): e22162404.*

A B S T R A C T

Acute Respiratory Infections (ARI) constitute an important health problem, since they are one of the first causes of medical attention worldwide, both in outpatient and inpatient consultations, and are among the first causes of mortality. The objective was to detect and identify viral agents causing ARI by molecular techniques in respiratory samples processed at the Molecular Biology laboratory of the Regional Hospital of Encarnación (HRE), Itapúa. A descriptive, cross-sectional, and quantitative study was conducted on 394 respiratory samples collected from April to August 2023, applying a multiplex panel by Real-Time Polymerase Chain Reaction (qPCR) panel. We analyzed variables such as sex, age, district of origin, clinical manifestations, Influenza-like Illness (ILI) or Severe Acute Respiratory Infection (SARI) and detected viral pathogen. Viruses were detected in 55.08% of samples. The most frequent viruses were Respiratory Syncytial Virus (RSV) (53.92%) and Rhinovirus (RV) (23.96%). The majority of patients with Viral Respiratory Infections (VRI) were children under 2 years of age (49.31%). RSV predominated in May and June in patients hospitalized for SARI (64.06%) and the districts with the highest frequency of VRI were Encarnación and Cambyreta (59.91% and 17.05%, respectively). RSV was the main cause of hospitalization for SARI in children under two years of age in Itapúa during May and June, highlighting the importance of prioritizing at-risk children and expanding studies to other etiologies.

Keywords: Respiratory Infections, Respiratory Sysncytial Viruses, Real-Time PCR, molecular diagnosis.

Identificación molecular de virus respiratorios en Itapúa, Paraguay durante el año 2023

R E S U M E N

Las Infecciones Respiratorias Agudas (IRA) constituyen un importante problema de salud, pues representan una de las primeras causas de atención médica en todo el mundo, tanto en la consulta ambulatoria como en la internación y se encuentran entre las primeras causas de mortalidad. El objetivo fue detectar e identificar agentes virales causantes de IRA por técnicas moleculares en muestras respiratorias procesadas en el laboratorio de Biología Molecular del Hospital Regional de Encarnación (HRE), Itapúa. Se realizó un estudio descriptivo, transversal y cuantitativo de 394 muestras respiratorias recolectadas entre abril y agosto del 2023, aplicando un panel multiplex por Reacción en Cadena de la Polimerasa en Tiempo Real (qPCR). Se analizaron variables como sexo, edad, distrito de procedencia; clínica, Enfermedad tipo influenza (ETI) o Infección Respiratoria Aguda Grave (IRAG) y patógeno viral detectado. En el 55,08% se detectó algún virus. Los virus más frecuentes fueron Virus Sincitial Respiratorio (VSR) (53,92%) y Rinovirus (RV) (23,96%). La mayoría de los pacientes con Infección Respiratoria Viral (IRV) fueron niños menores de 2 años (49,31%). El VSR predominó en los meses de mayo y junio en pacientes hospitalizados por IRAG (64,06% n= 89) y los distritos con mayor frecuencia de IRV fueron Encarnación y Cambyreta (59,91% y 17,05%, respectivamente). El VRS fue la principal causa de hospitalización por IRAG en menores de dos años en Itapúa durante mayo y junio, destacando la importancia de priorizar a los niños en riesgo y de ampliar los estudios a otras etiologías.

Palabras clave: Infecciones Respiratorias, Virus Sincitiales Respiratorios, PCR en Tiempo Real, diagnóstico molecular.

INTRODUCTION

Acute Respiratory Infections (ARI) are one of the primary causes of medical care worldwide and are among the first causes of mortality[1]. They are caused by viral, bacterial, fungal and parasitic, or by a combination of them, capable of causing clinical manifestations of varying complexity, such as Influenza-like Illness (ILI), up to more serious conditions manifested as Severe Acute Respiratory Infections (SARI)[2]. In children, respiratory infections caused by viral and bacterial agents are frequent, causing mostly nasopharyngitis, otitis, epiglottitis, laryngitis, bronchitis and pneumonia. Due to the development of new diagnostic methods and greater knowledge these types of diseases are acquiring greater importance[3-5].

Signs and symptoms of different pathogens causing ARI overlap, although seasonal patterns of virus activity may guide the etiological diagnosis. Rapid etiologic diagnosis improves patient management by enabling appropriate and timely therapy, reducing complications, symptom duration and hospital stay [6,7].

Nucleic Acids Amplification Techniques (NAAT) like Polymerase Chain Reaction (PCR) can be performed directly on the sample and allow the detection of more than one respiratory pathogen [6-10]. The diversity of microorganisms that can be detected, the need for a small sample volume, the sensitivity and specificity of the technique, the speed and ease with which it can be conducted, as well as the decreasing economic cost, ease the use of these methods[6].

Each year, an increase in the number of cases of viral respiratory infections (VRI) is found in the winter, in those countries that start this climatic season close to Epidemiological Week (EW) 16. VRI caused by Respiratory Syncytial Virus (RSV), Adenovirus (ADV), Influenza A and B and Parainfluenza (PIV) 1, 2, 3 and 4 are shown every year worldwide[11]. In Paraguay, the surveillance system detected an increase in the number of consultations for ILI and hospitalizations for SARI due to an increase in the activity of respiratory viruses, identifying co-circulation of RSV, Influenza A (H1N1), Influenza B and SARS-CoV-2 as of EW 14 (early April), with an increase in hospitalizations in children under 2 years and adults over 60 years[12].

The objective of this work was to detect and identify viral agents causing ARI by molecular techniques in patients attended at the Molecular Biology laboratory of the Regional Hospital of Encarnación (HRE), Itapúa from April to August 2023.

MATERIALS AND METHODS

We conducted a descriptive, cross-sectional and quantitative study of the viral pathogens causing respiratory infections detected in patients who underwent a respiratory panel by molecular detection in the Molecular Biology laboratory of the HRE from April to August 2023.

Respiratory samples (nasopharyngeal swabs) were processed by automated extraction of nucleic acids to detect and identify viral pathogens by Real Time PCR (qPCR) using Bosphore's Respiratory Pathogens Panel kit, which detects the following pathogens: Human Parainfluenza (PIV) 1,2,3 and 4; Human Enterovirus (hETV), Human Methapneumovirus (hMPV), Influenza A and B; Respiratory Syncytial Viruses (RSV), Human Bocavirus (hBoV), Rhinovirus (RV), Coronaviruses (CoV) (OC43, NL63; HKU1, 229E), Influenza A (H1N1), Human Parechovirus (HPeV) and Adenovirus (ADV).

The presence of one or more viral pathogens was reported as positive to the Central Public Health Laboratory (LCSP) and to the General Directorate of Health Surveillance (DGVS).

Epidemiological records were used to obtain the sociodemographic data of each patient. Access to the data was authorized by the director of the HRE and the research was approved by the Ethics Committee of the Autonomous University of Encarnación (UNAE).

A descriptive statistical analysis was performed with the freely available statistical program EpiInfoTM 7.2.5.0 and the evaluated variables were type of viral pathogen detected, patient's sex, age, district of origin and patient's clinical condition (ILI, SARI). We calculated the frencuency of VRI according to virus present, sex and age, frequency of pathogens per month. The geographical distribution of respiratory infections was set up according to the origin of the patients and their clinic records.

RESULTS

A total of 394 samples were analyzed, of which 204 belonged to women and 190 to men, ranging from children under 2 to adults over 60 years. Of these patients, 61.17% (n=241/394) were hospitalized for SARI and 38.3% (n=153/394) consulted for ILI. In 55.08% (n=217/394) one or more viruses causing respiratory symptoms were detected and in 44.92% (n= 177/394) none were detected.

The most often detected viruses were RSV (53.92% n=117) and RV (23.96% n=52).

In addition, co-detections between two or more viruses were seen, the most frequent being RSV with Influenza A (H1N1) (5.99% n=13) and RSV with RV (4.61% n=10) (Table 1).

Table 1. Frequency of viral agents detected by qPCR in the HRE starting from April to August of 2023.

Pathogen	Frequency (n=217)	Percentage (%)
RSV	117	53,92
RV	52	23,96
Influenza A (H1N1)	13	5,99
RSV, RV	10	4,61
Influenza B	5	2,30
CoV OC43	4	1,84
ADV	3	1,38
Influenza A	3	1,38
MPV	2	0,92
PIV 1	2	0,92
RV, CoV OC43	2	0,92
Influenza A, RV	1	0,46
MPV, RSV	1	0,46
RV, H. Influenza	1	0,46
RSV, CoV OC43	1	0,46

Most of the patients with some VRI were children under 2 years (Table 2). Female patients were more frequent (54.38% n=118/217).

Table 2. Frequency of VRI according to age range in the HRE starting from April to August of 2023.

Age range*	Frequency (n=217)	Percentage (%)
<2	106	49,31
2-4	36	16,59
5-19	35	16,13
20-39	22	10,14
40-59	5	2,30
60 and over	12	5,53

*Defined according to the General Directorate of Sanitary Surveillance of Paraguay.

About the clinical presentation, 64.06% (n= 139/217) who were hospitalized for SARI presented some etiologic agent of viral origin and 35.94% (n= 78/217) who consulted for ILI.

The distribution of viruses during the period from April to August showed frequencies of 6.45% (n=14) in April, 33.18% (n=72) in May, 47.93% (n=104) in June, 4.61% (n=10) in July and 7.83% (n=17) in August. In May and June, the virus with the highest circulation was RSV (19.82% (n=43) and 25.35% (n=55),

respectively) (Figure 1), in addition, consultations for ILI prevailed with 38.15% and hospitalizations for SARI increased in June by 53.24% (Figure 2).

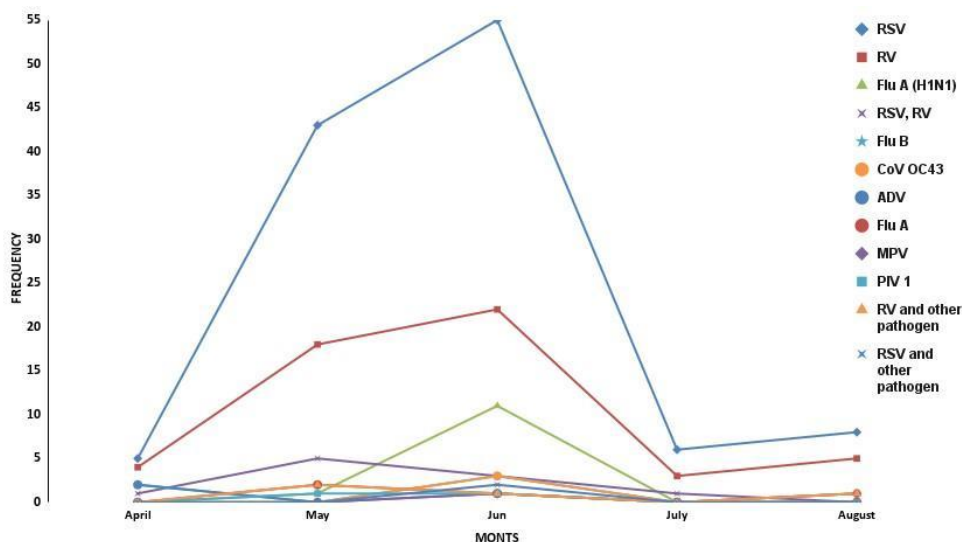*Reference:* Each virus is represented by a shape and a color.

Figure 1. Frequencies of viral pathogens (y-axis) detected by month (X-axis) in the HRE starting from April to August of 2023.
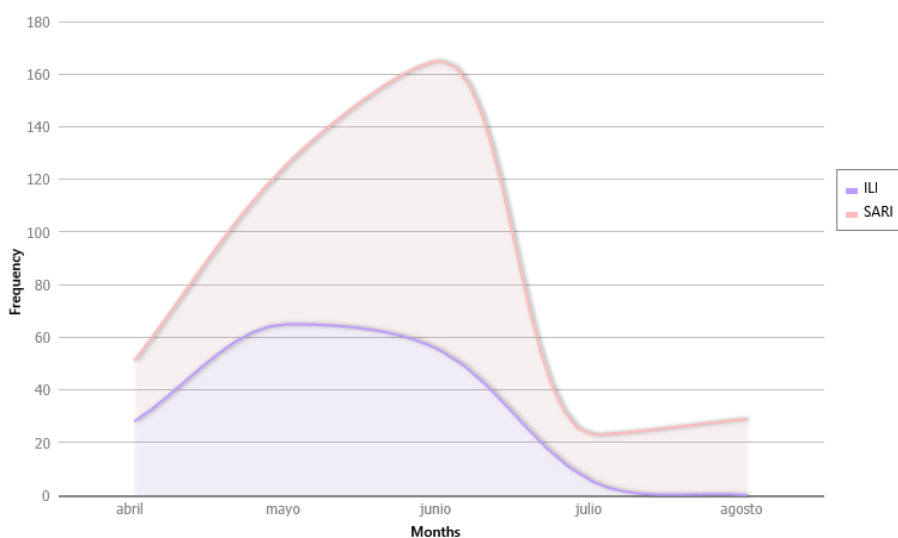

Figure 2. Frequency of consultations for ILI and hospitalizations for SARI in the HRE starting from April to August of 2023.

The patients who underwent the respiratory panel at the HRE came from the following 28 districts of the Department of Itapúa. Circulation of one or more viruses was detected in 22 districts, with Encarnación (59.91% n=130) and Cambyreta (17.05% n=37) having the highest frequency of IRV (Figure 3).
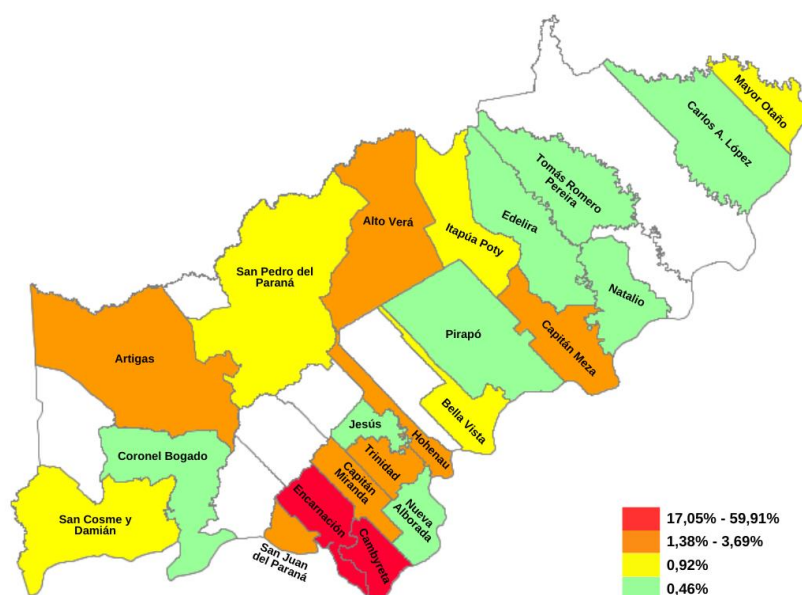
Figure 3. Geographical distribution of VRI in 22 districts belonging to the Department of Itapúa (map). The different districts are grouped in colors according to the frequency of detection.

DISCUSSION

During 2023, Argentina, Brazil, Uruguay, Chile, and Paraguay, experienced an early onset of the respiratory virus season, with a sharp increase in cases of ARI. In Argentina, there was an early increase in seasonal RSV and influenza A(H1N1) activity, with a low circulation of SARS-CoV-2 and other respiratory viruses. In Brazil, circulation of influenza and other respiratory viruses increased since February, with a higher number of hospitalized cases in children under 5 years due to RSV infection between May and July. Uruguay also experienced an increase in ARIs, with a higher and earlier peak in children under 15 years[13-16]. In Chile, there was a significant increase in ARIs associated with RSV in children under 1 year and in the 1–4-year group between April and May[17]. In Paraguay, there was an increase in consultations for ARI since January, which remained above the alert threshold, and a significant increase in respiratory viruses at the national level from May onwards[18].

In May and June in Paraguay, RSV was the predominant virus, affecting mainly children under 2 years of age and being the main cause of consultations and hospitalizations in this group. The increase in hospitalizations due to ARI was associated with RSV, with a higher incidence in the pediatric population, especially in children under 2 years of age. Amambay registered the highest number of hospitalizations due to RSV, followed by Alto Paraná and Central [19]. Itapúa had one of the highest number of hospitalizations due to RSV in May and June at the national level. ARI is an important cause of hospitalization in children under 2 years of age, with RSV being the main etiology[20]. At the end of June, there was a decrease in the demand for consultations for ILI at the national level and in the sentinel centers, as well as an increase in cases of Influenza A (H1N1) at the extremes of life[21]. In July, there was a slight decrease in demand for ILI consultations, but an increase in hospitalizations in sentinel centers[22]. In August, an increase in RV, ADV and MPV circulation was found, while RSV continued to be frequent in lower tract infections[23-25]. However, in Itapúa, RV was one of the most frequent viruses found in May and June, with a decrease in July and a slight increase in August.

In patients hospitalized for SARI who had evaluated negative to the panel of viral agents, it was not possible to know the causative agent of the clinic, since the qPCR technique only focused on finding viruses. This may be problematic, as bacterial infections may contribute to disease severity. Furthermore, no clear relationship

could be set up between virus co-detection and patients' clinical condition, as qPCR can detect viruses even in asymptomatic patients and the presence of multiple viruses may be due to various reasons, such as nosocomial infections, cocirculation of viruses or concurrent bacterial infections[26,27].

In conclusion, RSV was the most frequent viral pathogen in the department of Itapúa and the predominant causative agent of hospitalizations for SARI during May and June in patients under 2 years. This high incidence of RSV infection and its possible evolution and coinfection with other etiologic agents make it important to identify and prioritize children at higher risk of developing SARI associated with RSV. However, there were a large number of patients with SARI of unknown etiology, so it is recommended to extend the study to several etiologic agents.

The qPCR provides the possibility of finding the etiologic agents responsible for respiratory infections, which contributes to rapid clinical diagnosis, initial treatment and timely health strategies that prevent their spread and the unnecessary use of antibiotics.

Author contributions: Karina Cáceres Fernández: Conception/Design of the work, literature review, data analysis/discussion, preparation of the manuscript, revision of the final version. Ramona Morales Rivé: Data/information collection, preparation of the manuscript, revision of the final version. Sandra Caballero Gómez: Research conception/design, data/information collection.

Conflict of interest: This research does not present conflicts of interest.

REFERENCIAS BIBLIOGRÁFICAS

1. Estrada García CB, Recio Fornaris I, Martínez Orozco D, Collejo Rosabal YM, Mariño Serrano RY. Caracterización epidemiológica de las infecciones respiratorias agudas graves. Granma, marzo-mayo de 2020. Rev Med (Puebla). 2020; 24(6): 1242–57. Disponible en: http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S1028-48182020000601242

2. Vizzotti C, Tirado SM, Grinblat N, Molina V, Pagano I, Strella T, et al. Guía para la vigilancia epidemiológica y recomendaciones para la prevención y control de las infecciones respiratorias agudas. [Internet]. 2023 [citado el 3 de enero de 2024]. Disponible en: https://bancos.salud.gob.ar/sites/default/files/2023-05/guia-vigilancia-ira_2023.pdf

3. Margarita M, Milián L, Méndez López M, Méndez López L, Garaguey AN. Infecciones respiratorias agudas: breve recorrido que justifica su comportamiento. Rev Inf Cient. 2016; 95(2): 339–55. Disponible en: https://revinfcientifica.sld.cu/index.php/ric/article/view/114/1463

4. Martínez P, Pérez Martínez C, Rodríguez Toribio A, Jesús L, Martín P, Jesús J, et al. Comportamiento de las infecciones respiratorias agudas en un consultorio médico Behavior of acute respiratory infections in a medical office [Internet]. Vol. 14. 2018. Disponible en: https://revgaleno.sld.cu/index.php/ump/rt/printerFriendly/291/html

5. Pichler K, Assadian O, Berger A. Viral respiratory infections in the neonatal intensive care unit-a review. Front Microbiol. el 19 de octubre de 2018. Disponible en: https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2018.02484/full

6. Vila J, Gómez MD, Salavert M, Bosch J. Métodos de diagnóstico rápido en microbiología clínica: necesidades clínicas. Enferm Infecc Microbiol Clin. el 1 de enero de 2017; 35(1): 41–6. DOI: 10.1016/j.eimc.2016.11.004.

7. Sanbonmatsu-Gámez S, Pérez-Ruiz M, Lara-Oya A, Pedrosa-Corral I, Riazzo-Damas C, Navarro-Marí JM. Analytical performance of the automated multianalyte point-of-care mariPOC® for the detection of respiratory viruses. Diagn Microbiol Infect Dis. el 1 de noviembre de 2015; 83 (3): 252–6. DOI: 10.1016/j.diagmicrobio.2015.07.010.

8. Marimón JM, Navarro-Marí JM. Métodos de diagnóstico rápido de las infecciones respiratorias. Enferm Infecc Microbiol Clin [Internet]. el 1 de febrero de 2017 [citado el 8 de enero de 2024]; 35(2): 108–15. Disponible en: https://linkinghub.elsevier.com/retrieve/pii/S0213005X16303871

9. Díaz-Chiguer DL, Tirado-Mendoza R, Márquez-Navarro A, Ambrosio-Hernández JR, Ruiz-Fraga I, Aguilar-Vargas RE, et al. Detección y caracterización molecular de virus respiratorios causantes de infección respiratoria aguda en población adulta. Gac Med Mex. el 1 de junio de 2019; 155: S16–21. Disponible en:

https://doi.org/10.24875/gmm.19005138

10. Pérez-Ruiz M, Pedrosa-Corral I, Sanbonmatsu-Gámez S, Navarro-Marí JM. Laboratory Detection of Respiratory Viruses by Automated Techniques. Open Virol J. 2012; 7(1): 151–9. DOI: 10.2174/1874357901206010151

11. Véliz-Castro T, Mendoza KA, Arteaga Desireth YP, Clavijo Mereida J, Valero-Cedeño. Epidemiología de las infecciones respiratorias y sus factores predisponentes en adultos del cantón Jipijapa. Dom Cien. 2021; 7(4): 892–914. Disponible en: https://dialnet.unirioja.es/servlet/articulo?codigo=8383945

12. Vigilancia Universal IRAS y ETI. Planilla semanal de notificación obligatoria: VENOCD., Vigilancia Centinela de ETI e IRAG: Laboratorio Central de Salud Pública (LCSP), Laboratorios Regionales y de Hospitales Centinelas dependientes de la Red Nacional de Laboratorios. Alerta Epidemiologica Nro 5. 2023.

13. Organización Panamericana de la Salud (OPS), Organización Mundial de la Salud (OMS). Alerta epidemiológica Inicio anticipado del incremento de la actividad de virus respiratorios [Internet]. 2023. Disponible en: http://epi.minsal.cl/

14. Ministerio de Salud de Argentina. BOLETÍN EPIDEMIOLÓGICO NACIONAL. Semana Epidemiológica 36 [Internet]. 2023 sep. Disponible en: https://bancos.salud.gob.ar/sites/default/files/2023-09/ben-670_se37.pdf

15. Ministerio de Salud de Argentina. BOLETÍN EPIDEMIOLÓGICO NACIONAL. Semana Epidemiológica 37 [Internet]. 2023 sep. Disponible en: https://www.argentina.gob.ar/sites/default/files/2024/04/ben_670_se_37.pdf

16. Ministerio de Salud Argentina. Circulación de Influenza, VSR y otros virus respiratorios. 2023 jun. Disponible en: https://bancos.salud.gob.ar/sites/default/files/2023-06/comunicacion-influenza-ovr-06-2023.pdf

17. Ministerio de Salud de Chile. Informe virus respiratorios Campaña Invierno 2023 [Internet]. Santiago; 2023. Disponible en: https://www.minsal.cl/informe-virus-respiratorios-campana-invierno-2023/

18. Dirección General de Vigilancia Sanitaria (DGVS). Alerta Epidemiológica N° 4. Aumento de consultas por Enfermedad Tipo Influenza (ETI). 2023 abr. Disponible en: https://dgvs.mspbs.gov.py/wp-content/uploads/2023/05/Alerta-Epidemiologica-Nro-4_2023_final.pdf

19. Dirección General de Vigilancia Sanitaria (DGVS). Suben en un 15% las consultas por cuadros respiratorios. 2023. Disponible en: https://dgvs.mspbs.gov.py/suben-en-un-15-las-consultas-por-cuadros-respiratorios/

20. Lovera D, Peralta K, Gonzáles N, Amarilla S, Galeano F, Ayala J, et al. Vigilancia de Infecciones Respiratorias Agudas en niños y adolescentes en un Servicio de Pediatría. Revista del Instituto de Medicina Tropical. el 28 de diciembre de 2023;18(2):37–44. Disponible en: https://doi.org/10.18004/imt/2023.18.2.6

21. Dirección General de Vigilancia Sanitaria (DGVS). Disminuyen en un 20% las consultas por cuadros respiratorios. 2023. Disponible en: https://dgvs.mspbs.gov.py/disminuyen-en-un-20-las-consultas-por-cuadros-respiratorios/

22. Dirección General de Vigilancia Sanitaria (DGVS). Aumenta cifra de hospitalizados por infecciones respiratorias. 2023. Disponible en: https://dgvs.mspbs.gov.py/aumenta-cifra-de-hospitalizados-por-infecciones-respiratorias/

23. Dirección General de Vigilancia Sanitaria (DGVS). Consultas por enfermedades respiratorias muestran ligero aumento. 2023. Disponible en: https://dgvs.mspbs.gov.py/aumenta-cifra-de-hospitalizados-por-infecciones-respiratorias/

24. Dirección General de Vigilancia Sanitaria (DGVS). Demanda de consultas por cuadros respiratorios experimenta nuevo descenso. 2023. Disponible en: https://dgvs.mspbs.gov.py/demanda-de-consultas-por-cuadros-respiratorios-experimenta-nuevo-descenso/

25. Dirección General de Vigilancia Sanitaria (DGVS). Alerta Epidemiológica N° 7. Aumento de Consultas por Enfermedad Tipo Influenza (ETI). 2023 oct. Disponible en: https://dgvs.mspbs.gov.py/wp-content/uploads/2023/10/Alerta-epidemiologica-aumento-de-Consultas-por-ETI.pdf

26. Gil J, Almeida S, Constant C, Pinto S, Barreto R, Cristino JM, et al. Relevancia a corto plazo de la coinfección viral en pacientes menores de 2 años hospitalizados con infecciones de las vías respiratoriasinferiores. An Pediatr (Engl Ed). marzo de 2018; 88(3): 127–35. DOI: 10.1016/j.anpedi.2017.03.020

27. Esposito S, Daleno C, Prunotto G, Scala A, Tagliabue C, Borzani I, et al. Impact of viral infections in children with community-acquired pneumonia: results of a study of 17 respiratory viruses. Influenza Other Respir Viruses. el 13 de enero de 2013; 7(1): 18. DOI: 10.1111/j.1750-2659.2012.00340.x